

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
20 March 2003 (20.03.2003)

PCT

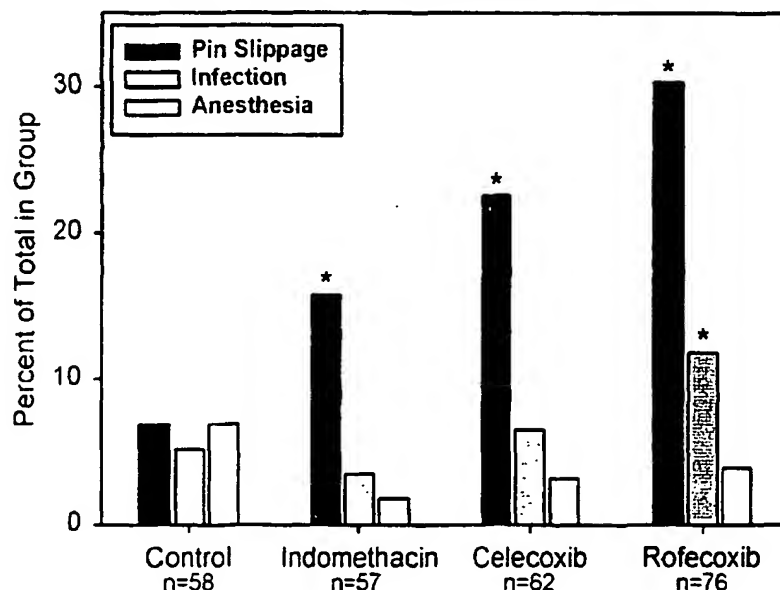
(10) International Publication Number  
**WO 03/022224 A2**

- (51) International Patent Classification<sup>7</sup>: **A61K**
- (21) International Application Number: PCT/US02/28930
- (22) International Filing Date:  
11 September 2002 (11.09.2002)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:  
09/953,067 11 September 2001 (11.09.2001) US
- (71) Applicant (for all designated States except US): **UNIVERSITY OF MEDICINE AND DENTISTRY OF NEW JERSEY [US/US]; 335 George Street, Suite 3200, New Brunswick, NJ 08901 (US).**
- (72) Inventor; and
- (75) Inventor/Applicant (for US only): **O'CONNOR, Patrick, J. [US/US]; 65 Waldon Road, Fanwood, NJ 07023 (US).**
- (74) Agent: **SLIGER, Lauren; Perkins Coie LLP, Patent-LA, P.O. Box 1208, Seattle, WA 98111-1208 (US).**
- (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- Published:  
— without international search report and to be republished upon receipt of that report

[Continued on next page]

(54) Title: COX-2 FUNCTION AND WOUND HEALING

Complication Rates Associated with NSAID Treatment during Fracture Healing (\* indicates  $P < 0.01$  based upon  $\chi^2$ )



(57) Abstract: The invention relates to compositions and methods for enhancing bone healing, bone formation and wound healing. More specifically, it relates to the use of cyclooxygenase 2 (COX-2) following bone fracture, orthopaedic procedure or wound infliction to enhance healing.

BEST AVAILABLE COPY



*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

## COX-2 Function and Wound Healing

### Field of the Invention

[0001] This invention relates to the field of cyclooxygenase activity and wound healing.

### 5 Background of the Invention

[0002] Bones, along with a small number of cartilages, comprise the skeletal system that serves as the rigid supporting framework of the body in adult humans. Certain parts of the supporting framework form chambers, such as the skull and the thoracic cage, that are important for protecting the soft parts contained in the  
10 chambers. Bones also serve as attachments for muscles and act as levers in the joint system of the body.

[0003] Mature bone is comprised of an organic framework of fibrous tissue and inorganic salts known as crystalline hydroxyapatite (HA). HA is composed of calcium and phosphorous, which are derived from the blood plasma and ultimately  
15 from nutritional sources. HA represents about 60 percent of the weight of compact bone and is deposited on a fibrous structure of collagenous connective tissue. Without HA, bone loses most its weight and rigidity and is susceptible to damage.

[0004] The process of bone formation, also known as osteogenesis, involves three main steps: production of the extracellular organic matrix (osteoid);  
20 mineralization of the matrix to form bone; and bone remodeling by resorption and reformation. The cellular activities of osteoblasts, osteocytes, and osteoclasts are essential to the process.

[0005] Osteoblasts synthesize the collagenous precursors of bone matrix and also regulate its mineralization. During bone formation, osteoblasts line tiny spaces known as lacunae within the surrounding mineralized matrix. Osteoblasts that line the lacunae are called osteocytes. Osteocytes occupy minute canals (canaliculi) which  
5 permit the circulation of tissue fluids. Hormones, growth factors, physical activity, and other stimuli act mainly through osteoblasts to bring about effects on bone. Osteoclasts are derived from hematopoietic stem cells that also give rise to monocytes and macrophages. Osteoclasts adhere to the surface of bone undergoing resorption and lie in depressions referred to as resorption bays. Osteoclasts are apparently  
10 activated by signals from osteoblasts. Osteoclastic bone resorption does not occur in the absence of osteoblasts. To meet the requirements of skeletal growth and mechanical function, bone undergoes dynamic remodeling by a coupled process of bone resorption by osteoclasts and reformation by osteoblasts.

[0006] Bone is formed through one of two pathways, by replacement of  
15 cartilage or by direct elaboration from periosteum. These processes are known, respectively, as endochondral ossification and intramembranous ossification. During endochondral ossification, a cartilaginous bone model is first formed. Then, a layer of bone on the surface of the cartilaginous shaft is formed by osteoblasts. Succeeding layers of bone follow. At the same time, the matrix of cartilage cells is calcified into  
20 a trabecular network of cartilage while the interstitial cartilage is degenerated. The combined processes of calcification and degeneration of the cartilage advance from the center toward the ends of the cartilage model. The osteoblasts penetrate the cartilage along with capillaries to produce bone on the cartilaginous trabeculae and



advance from the center to the ends to progressively form bone on the cartilaginous trabeculae. Ultimately, the calcified cartilage is completely replaced by spongy bone.

[0007] In contrast, the process of intramembranous ossification does not involve a cartilaginous template. Instead, mesenchymal cells become osteoblasts which begin to form the branching trabeculae of bone. The initial thin trabeculae are some times referred to as spicules. The trabecular bone becomes denser by widening of the trabeculae, and is then remodeled externally and internally. The mandibles, clavicles and certain bones of the skull are produced through intramembranous ossification.

10 [0008] There are a number of diseases related to bone formation, deterioration and healing, including osteoporosis, osteogenesis imperfecta (OI) and fibrodysplasia ossificans progressiva (FOP). Osteoporosis, or porous bone, is a disease characterized by low bone mass and structural deterioration of bone tissue, leading to bone fragility and an increased susceptibility to fractures of the hip, spine, and wrist. Osteoporosis  
15 is a major public health threat for more than 28 million Americans, 80 percent of whom are women. The strength of bone depends on its mass and density. Bone density depends in part on the amount of calcium, phosphorus and other minerals bones contain. Bones that contain less mineral are weakened and lose internal supporting structure. A full cycle of bone remodeling takes about 2 to 3 months.  
20 Children tend to make new bone faster than old bone is broken down. As a result, bone mass increases. Peak bone mass is reached in an individual's mid-30s. Although bone remodeling continues, old bone is broken down faster than new bone is formed. As a result, adults lose slightly more bone than is gained - about 0.3

percent to 0.5 percent a year. Lack of vitamin D and calcium in an individual's diet can accelerate the process. In addition, for women at menopause, estrogen levels drop and bone loss accelerates to about 1 percent to 3 percent a year. Bone loss slows but doesn't stop at around age 60. Women may lose between 35 percent and 50 percent of their bone mass, while men may lose 20 percent to 35 percent of their bone mass. Development of osteoporosis depends on the bone mass attained between ages 25 and 35 (peak bone mass) and how rapidly it is lost as an individual get older. The higher an individual's peak bone mass, the less likely that individual will develop osteoporosis. Calcium, vitamin D and exercising regularly are important for maintaining bone strength. Nonetheless, methods for effectively treating osteoporosis are still desired.

[0009] Osteogenesis Imperfecta (OI) is a genetic disorder characterized by bones that break easily, often from little or no apparent cause. There are at least four distinct forms of the disorder, representing extreme variation in severity from one individual to another. For example, a person may have as few as ten or as many as several hundred fractures in a lifetime. While the number of persons affected with OI in the United States is unknown, the best estimate suggests a minimum of 20,000 and possibly as many as 50,000. OI can be dominantly or recessively inherited and can also occur as a mutation. A cure for OI has not yet been discovered. As a result, methods for treatment focus on preventing and controlling symptoms, strengthening bone mass and ensuring proper healing.

[0010] In addition, both osteoporosis and OI leave patients vulnerable to bone fractures. If these bone fractures do not heal properly, these patients may continue to

suffer from pain and may be at increased risk for further fractures as well as other related complications. Methods or treatments that enhance and/or ensure proper fracture healing are important for patients with osteoporosis or brittle bone disease. Another group that will benefit from methods for enhanced wound healing are the elderly and patients who have undergone orthopaedic procedures.

[0011] Fracture healing is the culmination of a highly orchestrated series of physiological and cellular pathways to restore the function of broken bones. Fracture healing generally involves the following steps: the formation of a hematoma (collection of blood at the fracture site), development of a soft callus due to cell multiplication in the lining of the injured bone, growth of blood vessels and fibrocartilage in the middle of the fracture, formation of osteoblasts that migrate into the callus and deposit calcium to form a hard callus, and remodeling and strengthening of the bone through osteoblast and osteoclast formation.

[0012] Osteogenesis during fracture healing occurs by intramembraneous and endochondral ossification that histologically resembles fetal skeletogenesis (Einhorn 1998; Vortkamp et al. 1998; Ferguson et al. 1999). However, the localized tissue hypoxia, the fracture hematoma, subsequent inflammation at the fracture site, and the frank remodeling of the fracture callus at the later stages of healing are unique physiological and cellular responses to bone fractures that have no known corresponding counterpart during fetal development of the skeleton.

[0013] It has been hypothesized that the early physiological responses to a bone fracture, namely hypoxia and inflammation, induce gene expression pathways and promote cell proliferation and migration into the fracture site in order to promote

healing (Brighton et al. 1991; Bolander 1992). Production or release of specific growth factors, cytokines, and local hormones at the fracture site by these physiological processes would create the appropriate microenvironment to (1) stimulate periosteal osteoblast proliferation and intramembraneous ossification to form the hard fracture callus, (2) stimulate cell proliferation and migration into the fracture site to form the soft callus, and (3) stimulate chondrocyte differentiation in the soft callus with subsequent endochondral ossification. Remodeling of the fracture callus by osteoclastic resorption and subsequent osteogenesis converts the fracture callus woven bone into cortical bone and thereby restores the shape and mechanical integrity of the fractured bone.

[0014] One potential class of factors that would mediate certain events of fracture healing is the prostaglandins. The effects of prostaglandins on bone metabolism are complex since prostaglandins can stimulate bone formation as well as bone resorption (Kawaguchi et al. 1995). However, because the in vivo half-life of purified or synthetic prostaglandins is very short, prostaglandins per se have a limited therapeutic value.

[0015] Prostaglandins are synthesized by osteoblasts and different cell stimuli can alter the amount and possibly the spectrum of prostaglandins produced by osteoblasts (Feyen et al. 1984; Klein-Nulend et al. 1997; Wadleigh and Herschman 1999). Therefore, signal transduction, mechanical perturbations, or other physiological signals can affect bone metabolism through alteration of prostaglandin production.

[0016] Prostaglandin synthesis begins with the release of arachidonic acid from membrane phospholipids by phospholipase activity. Arachidonic acid is subsequently converted into prostaglandin  $H_2$  ( $PGH_2$ ) by cyclooxygenase (COX) via two independent catalytic steps (Needleman et al. 1986). Synthase enzymes then  
5 convert  $PGH_2$  into the specific prostaglandins produced by that cell such as  $PGD_2$ ,  $PGE_2$ ,  $PGF_{2\alpha}$ , prostacyclin, and thromboxane. Thus, cyclooxygenase activity is essential for normal prostaglandin production and cyclooxygenase is believed to be the rate-limiting enzyme in the prostaglandin synthetic pathway.

[0017] There are two known forms of cyclooxygenase, COX-1 and COX-2,  
10 which are encoded by two genes (Xie et al. 1991; O'Banion et al. 1992). COX-1 is constitutively expressed by many tissues and provides a homeostatic level of prostaglandins for the body and specific organs, such as the stomach and kidneys (Vane et al. 1998). In contrast, COX-2 is inductively expressed in vitro by a diverse array of cell stimuli such as exposure to lipopolysaccharide (O'Sullivan et al. 1992a;  
15 O'Sullivan et al. 1992b), certain cytokines and growth factors (O'Banion et al. 1992; Wadleigh and Herschman 1999), or mechanical stress (Topper et al. 1996; Klein-Nulend et al. 1997). COX-2 expression can be stimulated in vivo by wounding and inflammation (Masferrer et al. 1994; Shigeta et al. 1998; Muscará et al. 2000).

[0018] Inhibiting the cyclooxygenase activity of COX-1 and COX-2 can  
20 reduce prostaglandin synthesis by preventing the conversion of arachidonic acid into  $PGG_2$ , the precursor of  $PGH_2$ . This is commonly done to reduce inflammation and pain with aspirin and non-steroidal anti-inflammatory drugs (NSAIDs), such as indomethacin. Most NSAIDs inhibit the cyclooxygenase activity of COX-1 and

COX-2 with near equal potency, which often leads to detrimental gastro-intestinal or kidney side effects (Raskin 1999; Whelton 1999). Use of COX-2-selective NSAIDs has become very popular since these drugs, such as celecoxib (*Celebrex*) and rofecoxib (*Vioxx*) preferentially inhibit the cyclooxygenase activity of COX-2 with  
5 selectivity relative to COX-1 of approximately 8-fold for celecoxib and 35-fold for rofecoxib (Riendeau et al. 2001).

[0019] Prostaglandins are produced during fracture healing. Prostaglandin levels in and around the healing callus of rabbit tibia that had been severed by osteotomy showed that PGE and PGF levels were elevated between 1 and 14 and 7  
10 and 14 days post-osteotomy, respectively (Dekel et al. 1981). No survey of the temporal pattern or variety of prostaglandins produced during fracture healing has been reported for other rodents or man. Non-specific NSAIDs have been shown to delay but not stop fracture healing in experimental animal models (Rø et al. 1976; Allen et al. 1980; Altman et al. 1995). In addition, non-specific NSAIDs have been  
15 shown to reduce the incidence and severity of heterotopic (abnormal or deviating from the natural position) bone formation in humans following certain fractures or orthopaedic surgical procedures (Pritchett 1995; Moore et al. 1998). These observations suggest that prostaglandins are necessary for bone formation but given the limitations of non-specific NSAID use, it is unknown whether prostaglandins  
20 produced by COX-1, COX-2 or both enzymes are essential for fracture healing.

### Summary of the Invention

[0020] The present invention relates to compositions and methods for use in wound healing and for use in enhancing fracture healing, bone formation and wound

healing. The present invention further provides for methods for treating diseases related to bones, including osteoporosis, osteogenesis imperfecta and fibrodysplasia ossificans progressiva.

[0021] One embodiment of the present invention involves a vector for use in wound healing comprising a promoter linked to a cyclooxygenase expression cassette. In a further embodiment, the vectors of this invention may be used in gene therapy approaches to enhance wound healing. The wound conditions of this invention can include, bone fractures and skin lesions. The methods of this invention are particularly useful for wound healing in the elderly, patients with osteoporosis and OI, and patients that suffer from delayed wound healing.

[0022] In another embodiment, cyclooxygenase proteins, including COX-1, COX-2 or a combination of the two, are formulated as pharmaceutical compositions for use in wound healing. In a further embodiment of the invention, the pharmaceutical compositions are combined with a carrier for applications in wound healing.

[0023] Another embodiment of the invention provides for the use of the vectors of this invention in gene therapy approaches and/or the pharmaceutical compositions to treat osteoporosis, OI and other related brittle bone conditions. In a further embodiment, the vectors and compositions are used therapeutically to counteract conditions associated with osteoporosis, OI and brittle bones conditions. In a further embodiment, the vectors and compositions are used for wound healing and/or to enhance wound healing in patients with osteoporosis, OI or brittle bone conditions.

[0024] In another embodiment of the invention, COX-2 selective NSAIDs are used in the treatment of heterotopic ossification conditions. Such conditions can include fibrodysplasia ossificans progressiva. In addition, heterotopic ossification can occur following hip replacement procedures and after acetabular fractures. COX-2  
5 selective NSAIDs can be used to treat heterotopic ossification under these circumstances as well.

### **Brief Description of the Drawings**

#### **Figure 1. Radiographic Analysis of Fracture Healing in NSAID Treated Rats**

[0025] High resolution radiographs were made immediately post-fracture and  
10 then every week till the endpoint of the experiment (8 weeks) using a Hewlett-Packard Faxitron. Shown are radiographs of the fractured right femurs from the same rats taken at 2 (top), 4 (middle), and 8 weeks (bottom) post-fracture (dorsal-ventral view). As indicated, panels A-D shows radiographs from a no drug rat, an indomethacin treated rat, a celecoxib treated rat, and a rofecoxib treated rat,  
15 respectively. Note that the fracture is still clearly evident in the 8-week post-fracture radiographs of the celecoxib and rofecoxib treated rats.

#### **Figure 2. COX-2-Selective NSAIDs Alter the Mechanical Properties of Fractured Femurs**

[0026] Mechanical testing data was obtained or derived as described in the  
20 Experimental Procedures. The data for each fractured femur was normalized as a percentage of the value obtained from that animal's contralateral, unfractured femur,



except for shear modulus (panel D). Shown are the mean normalized values at each time point and for each treatment group or the mean shear modulus values in panel D. The error bars represent standard errors of the mean. Pairwise t-test were made between the no drug and experimental treatments within a time point. Statistical significance differences ( $P < 0.05$ ) are noted with asterisks.

**Figure 3. COX-2-Selective NSAIDs Disrupt Endochondral Ossification During Fracture Healing**

[0027] Shown are fracture calluses from no drug, indomethacin, celecoxib, and rofecoxib treated rats at 2, 3, and 4 weeks post-fracture as indicated. The specimens were embedded in polymethylmethacrylate (PMMA), sectioned, and stained with van Gieson's picrofuchsin and Stevenel's blue so that bone is red, calcified cartilage is orange to red, cartilage is deep blue to purple, and fibrous tissue and muscle is pale blue. Each section is oriented with the cortical bone on the bottom, fracture callus on top, and fracture site in the middle. Note the abnormal cartilage morphology in the calluses of the NSAID treated rats (panels D, G, and J) and the lack of cartilage in the celecoxib (panels H and I) and rofecoxib treated (panels K and L) rats at 3 and 4 weeks post-fracture.

**Figure 4. Abnormal Bone Resorption during Fracture Healing in COX-2-Selective NSAID Treated Animals**

[0028] Shown are fracture calluses from rofecoxib treated rats at 3 (panels A and B) and 4 weeks (panel C) post-fracture. The orientation of panels A and B are the same with external callus on top and fracture site to the immediate left of the panel.

In panel C, the external callus is to the left and the fracture site is at the immediate bottom of the panel. The specimens were embedded in PMMA, sectioned, and stained with van Gieson's picrofuchsin and Stevenel's blue so that bone is red, calcified cartilage is orange to red, cartilage is deep blue to purple, and other cell types are shades of blue. Original photographic magnification is indicated. **CB**: cortical bone; **WB**: woven bone; **CC**: calcified cartilage; **Ca**: cartilage; **F**: fracture site; **ab**: air bubble; **M**: area of magnification shown in panel B; and **Oc**: osteoclasts. The NSAID treated rats often developed areas of high bone resorption at the cortical bone, fracture site, external callus junction (**M**) as seen in panel A. The air bubble (ab) seen in panel A is an artifact of the PMMA embedding. At higher magnification, osteoclasts (**Oc**) can be seen lining the cortical bone surface of area **M** in the 3 week fracture callus as denoted by the arrows. Shown in panel C is an identical area of a 4 week post-fracture callus as shown in panel B. The extent and area of bone resorption appears to be greater at 4 weeks post-fracture and also often encompassed all surfaces of the cortical bone at the fracture site. Similar bone resorption patterns were seen in celecoxib treated rats and to a lesser extent in the indomethacin treated rats.

### **Figure 5. Experimental Complications Associated with NSAID Treatment during Fracture Healing**

[0029] Complications that necessitated the pre-mature euthanization or resulted in the pre-mature death of a rat during the course of these experiments were compiled and used to determine the effects of NSAID treatment on anesthetic death, infection, and pin slippage. Experimental treatment group values were compared to the no drug values using a  $\chi^2$  analysis. Significant differences are noted with an

asterisk ( $P < 0.01$ ). As can be seen, pin slippage was by far the most common complication and was significantly different for each treatment group relative to the no drug rats with P-values of less than  $1E^{-4}$ ,  $1E^{-7}$ , and  $1E^{-24}$  for the indomethacin, celecoxib, and rofecoxib treated rats respectively. The rofecoxib treated rats were also found to have a statistically significant higher infection rate as compared to the no drug rats ( $P < 0.0001$ ). Death from anesthesia was not different between groups.

#### Figure 6. *Cox2* but not *Cox1* is Essential for Normal Bone Fracture Healing

[0030] The right femora of *Cox1*<sup>-/-</sup> and *Cox2*<sup>-/-</sup> mice were fractured and examined radiographically and histologically at 2 weeks post-fracture. Panels A and D. Radiographs of fractured femurs from a *Cox1*<sup>-/-</sup> and a *Cox2*<sup>-/-</sup> mouse, respectively. Note the lack of mineralized tissue (X-ray dense) in the fracture callus region of the *Cox2*<sup>-/-</sup> mouse. Panels B and C. Sagittal section through the fractured femur of a *Cox1*<sup>-/-</sup> stained with Masson's trichrome stain (cell nuclei = purple; muscle and cytoplasm = red; collagen and bone = blue). Panels E and F. Sagittal section through the fractured femur of a *Cox2*<sup>-/-</sup> stained with Masson's trichrome stain. Note the presence of chondrocytes within the *Cox2*<sup>-/-</sup> callus but the lack of endochondral ossification relative to the *Cox1*<sup>-/-</sup> mouse fracture callus. Original photographic magnification is indicated. The *Cox2*<sup>-/-</sup> mouse fracture callus specimens are shown at higher magnification because the callus was smaller. B: bone; C: chondrocytes and cartilage; E: area of endochondral ossification; M: area of intramembraneous bone formation; F: fracture site.

### Detailed Description of the Invention

[0031] The present invention involves methods for enhancing wound healing and treating conditions of the bone with the use of cyclooxygenase (COX). COX is an enzyme that converts arachidonic acid into prostaglandin  $H_2$  (PGH<sub>2</sub>). PGH<sub>2</sub> is then converted into specific prostaglandins, which affect bone metabolism and formation. There are two forms of cyclooxygenase, COX-1 and COX-2. COX-1 is constitutively expressed, while COX-2 is inductively expressed by various stimuli. Examples of such stimuli are wounding and inflammation. The subsequent expression of COX-2 in response to these stimuli results in the production of pro-inflammatory prostaglandins. The pain and inflammation associated with a wound are commonly treated with non-steroidal anti-inflammatory drugs (NSAIDs). Most NSAIDs inhibit the cyclooxygenase activity of both COX-1 and COX-2. However, due to the inhibition of COX-1 activity, gastro-intestinal and kidney side effects result from NSAIDs use. COX-2 selective NSAIDs have also been developed which preferentially inhibit COX-2 activity relative to COX-1 activity and thereby avoid the side effects associated with non-specific NSAIDs.

[0032] In addition to its inflammatory function, one aspect of the invention relates to the necessity of COX-2 activity in wound healing. As described in more detail below, COX-2 is essential for normal wound healing. In another aspect of the invention, treatment systems and methods employ COX-2 to enhance wound healing.

[0033] In a preferred embodiment of the invention, gene therapy techniques are employed to increase cyclooxygenase activity at the site of the wound to enhance wound healing. Gene therapy techniques allow an absent or faulty gene to be

replaced with a working gene. They also allow for the delivery and controlled expression of therapeutic gene products. One embodiment of the invention provides for a vector containing a cyclooxygenase expression cassette. In a further embodiment, the vector containing the cyclooxygenase expression cassette is  
5 delivered to the wound using gene therapy techniques. The gene therapy techniques may use adenoviral vectors, adeno-associated viral vectors, recombination-defective retrovirus vectors or DNA vectors to deliver the cyclooxygenase expression cassette to the wound.

[0034] The vectors of this invention may be used to increase cyclooxygenase  
10 levels at the site of the wound and thereby enhance wound healing. For example, these vectors may be used to enhance wound healing following bone fractures or orthopaedic procedures. The vectors may also be of particular use to the elderly and other patient groups that have delayed bone healing, including smokers, diabetics, and steroid users. In addition, the vectors may be used in the treatment of osteoporosis  
15 and osteogenesis imperfecta using gene therapy techniques for gene replacement and to enhance healing for wounds resulting from these diseases.

[0035] One aspect of the invention involves a vector that contains a cyclooxygenase expression cassette that encodes a cyclooxygenase gene product. The vector includes all necessary sequences for the expression of the cyclooxygenase  
20 expression cassette and any sequences that may be included to control the expression of the cassette. These sequences may include, but are not limited to, a promoter or initiation sequence, an enhancer sequence, termination sequence, RNA processing signals, and/or a polyadenylation signal sequence.

[0036] The term “vector” refers to a nucleic acid construct that encodes for a particular gene product. The vectors of the present invention are preferably adenoviral, adeno-associated viral, recombination-defective retrovirus, plasmid or DNA vectors.

5 [0037] The term “cyclooxygenase expression cassette” refers to nucleic acid which codes for the cyclooxygenase enzyme, preferably COX-1 or COX-2. The expression cassette is positioned within the vector such that it can be transcribed into RNA and translated into the cyclooxygenase protein product. Table 3 provides for the human cDNA sequences for COX-1 (SEQ. ID. NO. 1) and COX-2 (SEQ.ID. NO. 2).

10 [0038] The term “necessary sequences for the expression of cyclooxygenase” refers to sequences necessary to ensure the transcription and translation of the expression cassette. The term “promoter” refers to a DNA sequence that is bound by RNA polymerase and is required to initiate transcription of a gene. There are a number of promoters that are known in the art, including those that can enhance or  
15 control expression of the gene or expression cassette. For example, cytomegalovirus promoter may be fused to the cyclooxygenase expression cassette to obtain constitutive expression of cassette or a COX-2 promoter may be linked to a cyclooxygenase cDNA to allow for expression of cyclooxygenase in a more normal fashion. For example, a COX-2 promoter may be linked to a COX-1 cDNA. This  
20 may allow a patient to remain on COX-2 selective NSAIDs but still have the ability to heal. In another aspect of the invention, the promoter may be induced in response to inflammation. Preferably, the inducible promoter employs the use of inflammatory gene promoters such as the interleukins, in particular, the IL-6 promoter, or the

complement system gene promoters. Other inflammatory gene promoters include promoters for TNF- $\alpha$ , or the NF- $\kappa$ B response element.

[0039] In another aspect of the invention, the vectors are delivered directly to the location of the wound by injection or direct application in order to enhance wound healing. The vectors of this invention may also be administered by electroporation or delivered as an aerosol. The vectors may be administered or delivered in saline solutions, encapsulated in liposomes, in polymer solutions, in gels or lyophilized. In an alternative aspect, targeted transfection may be used to deliver the vectors in vivo. The term "wound" refers to a bone fracture, the site of a surgical orthopaedic procedure, or a skin lesion. In another aspect of the invention, the vectors may be delivered ex vivo, wherein a patient's cells are transfected ex vivo with the vectors of this invention and the transfected cells are then returned to the patient.

[0040] A further aspect of this invention provides for pharmaceutical compositions and methods of their use in wound healing. These pharmaceutical compositions include cyclooxygenase protein formulations and/or pharmaceutically acceptable carriers. The cyclooxygenase protein formulations comprise COX-1 protein, COX-2 protein or a combination of the two that are formulated such that the proteins remain stable, retain their function, including their enzymatic activity, and are physiologically acceptable. The human amino acid sequences for COX-1 (SEQ. ID. NO. 3) and COX-2 (SEQ. ID. NO. 4) proteins are provided in Table 3. There are a variety of pharmaceutically acceptable carriers that are known in the art, including, but not limited to, saline, liposomes, gels and polymers. The formulation and/or carrier may also be lyophilized for aerosol delivery. In a further embodiment of the

invention, these pharmaceutical compositions are administered topically, orally, intravenously, nasally or via inhalation, or locally for use in wound healing. Preferably, these composition are administered to enhance wound healing.

[0041] In another aspect of the invention, methods of enhancing wound healing in the elderly are provided. COX-2 gene expression is reduced with age as are wound healing and bone formation. In one embodiment, vectors containing cyclooxygenase expression cassettes are administered to elderly to enhance would healing by increasing COX-2 expression during wound healing. A further aspect of this invention provides for methods for enhancing wound healing in patient groups that have delayed wound healing by delivering the vectors of this invention to these patients during wound healing.

[0042] In another embodiment of the invention, the vectors of this invention are used to enhance wound healing in patients suffering from osteoporosis or OI by employing gene therapy techniques to deliver cyclooxygenase expression cassettes to the wound to enhance healing.

[0043] In another aspect of the invention, a method for treating pathological heterotopic ossification conditions is provided. Pathological heterotopic ossification conditions are diseases characterized by abnormal bone formation at locations of inflammation. An example of a pathological heterotopic ossification condition is fibrodysplasia ossificans progressiva (FOP). In one embodiment of the invention, COX-2 selective NSAIDs are administered to locations of inflammation that lack a wound. The COX-2 selective NSAIDs inhibit COX-2 activity thereby preventing abnormal bone formation.



[0044] Our experimental approach was to assess fracture healing using a standard rat closed femur fracture model in which COX-2 function was inhibited in vivo with the COX-2-selective NSAIDs, celecoxib and rofecoxib. The results were striking in that femur fracture healing in rats treated with celecoxib or rofecoxib was dramatically impaired. These observations were confirmed by examining fracture healing in *Cox1* and *Cox2* null mice. Histological observations indicated that the defect in fracture healing caused by the COX-2-selective NSAIDs or by lack of *Cox2* occurred in the endochondral ossification pathway.

[0045] Using a standard rat closed femur fracture model, we have demonstrated that COX-2-selective NSAID treatment can stop normal fracture healing and induce the formation of mal-unions and non-unions. Non-unions refer to fractures that show no visible sign of healing. Mal-unions refer to bones that do not heal properly and result in misalignment of bones. These observations are in grim contrast to those observations we, and others have obtained by treating rats with non-selective NSAIDs such as indomethacin when it was observed that fracture healing was delayed but not prevented (Rø et al. 1976; Rø et al. 1978). Our observations suggest that COX-2 has an essential function during normal fracture healing and that COX-2-selective NSAID inhibition of prostaglandin synthesis stops normal fracture healing. This also suggests that the inflammatory phase is critical for normal fracture healing.

[0046] Consistent with the effects of COX-2-selective NSAIDs on rat femur fracture healing, mice homozygous for a targeted mutation in *Cox2*, but not *Cox1*, also showed inhibited fracture healing (Figure 6). The cDNA sequences (SEQ. ID.

NO. 5 for COX-1 and SEQ. ID. NO. 6 for COX-2) and corresponding amino acid sequences (SEQ. ID. NO. 7 for COX-1 and SEQ. ID. NO. 8 for COX-2) for mouse COX-1 and COX-2 gene and protein are provided in Table 4. This excludes the possibility that any additional inhibitory activity against other cellular processes or proteins by celecoxib or rofecoxib (Jones et al. 1999; Hsu et al. 2000; Rossi et al. 2000) is primarily responsible for negatively affecting fracture healing.

[0047] The amount of rofecoxib used to treat the rats (3mg/kg) in this study was approximately 4 times the nominal, maximum human daily dose of 50mg (0.7mg/kg) that is used to manage acute pain. In contrast, the celecoxib dose used to treat rats in this study was in the recommended dose range for humans. The rats received 280mg/70kg body weight of celecoxib once per day whereas the recommended human maximum daily dose of celecoxib is 200mg twice a day. Additionally, the rats in this study received daily doses of each drug till the endpoint of the experiment, which is unlike common clinical scenarios when COX-2-selective NSAIDs are used to acutely manage pain, inflammation, and swelling following a fracture. In contrast, arthritis patients, in particular, do use COX-2-selective NSAIDs on a daily basis for extended periods. Based simply upon animal body weight and drug dose, and without accounting for pharmacokinetic variables, the indomethacin dose used (1mg/kg) would be predicted to inhibit most COX-1 activity but to only partially inhibit COX-2 activity; the celecoxib dose used should inhibit most if not all COX-2 activity and possibly inhibit some COX-1 activity; and the rofecoxib dose used should completely inhibit COX-2 activity but not affect COX-1 (Warner et al. 1999). The estimated plasma half-lives for celecoxib and rofecoxib in male rats

following a single drug dose are approximately 4 and 5 hours respectively (Halpin et al. 2000; Paulson et al. 2000). In humans, however, the estimated elimination half-life for celecoxib is 11 hours (Davies et al. 2000) and the plasma half-life for rofecoxib is approximately 10-17 hours depending upon drug dose (Depré et al. 5 2000). Unfortunately, these data were unknown to us when this study was initiated and consequently our rat drug dosing regime may actually be an under representation of the COX-2 inhibition level over a 24 hour cycle as that for humans receiving similar drug doses. Additionally, celecoxib and rofecoxib may have different inhibitory concentrations for rat versus human COX-2. Despite the pharmacokinetic 10 variations between rats and humans receiving COX-2-selective NSAIDs, our data clearly indicate that these drugs have a dramatic negative effect on fracture healing in mammals, and thus caution in the use of these COX-2-selective NSAIDs in humans is warranted.

[0048] The fractured femurs of the celecoxib treated rats had increased 15 mechanical properties and healed as mal-unions, rather than non-unions, in approximately 50% of the rats tested (Tables 1 and 2). Had the experimental endpoint been extended, some of the celecoxib treated rats may have gone on to heal their femur fractures. However, the delay in any such healing among the celecoxib treated rats would have been significantly longer than in no drug or indomethacin treated rats.

20 [0049] After mechanically testing the celecoxib and rofecoxib treated fractured femurs, we observed that the fracture callus had a shell-like morphology. The periphery of the fracture callus was bone that sometimes partly bridged the fracture gap and thus formed a mal-union in the celecoxib treated rats. However, little

or no bone was present between the peripheral bone of the callus and the original femoral cortical bone ends. Often this space appeared to be filled with fatty marrow. Also strikingly apparent was the lack of new bone or primary bone healing at the cortical bone ends.

- 5    **[0050]**       The indomethacin dose used in this study (1 mg/kg) was previously shown to delay fracture healing in rats and was used principally as a positive control (Altman et al. 1995). Increasing indomethacin doses to levels that which would completely inhibit COX-2 (and COX-1 activity) causes a steep increase in rat mortality from gastrointestinal bleeding (Allen et al. 1980; Wallace et al. 1998).
- 10   Consequently, it would be difficult to directly compare the effects of COX-2-selective and traditional NSAIDs on fracture healing based solely upon COX-2 inhibition levels. An alternative approach would be to measure prostaglandin levels within and around the fracture callus during healing in control and drug treated rats and to then correlate those observations with different healing parameters.
- 15   **[0051]**       The abnormal osteoclastic response observed at the fracture site in the NSAID treated rats is counter to experimental observations in which prostaglandins stimulate bone resorption (Klein and Raisz 1970) and COX-2 function promotes osteoclast formation (Okada et al. 2000). Lack of prostaglandins in the fracture callus could induce osteoclastic activity through an undescribed mechanism, or perhaps
- 20   through an indirect mechanism such as increased mechanical instability at the fracture site. An additional possibility is that the amount and/or repertoire of prostaglandins produced at the fracture site in the NSAID treated rats is competent for inducing osteoclastic activity but insufficient for osteogenesis to proceed normally. The second

possibility is favored since the relative short half-life of celecoxib and rofecoxib in rats should produce daily periods in which COX-2-dependent prostaglandin synthesis could occur and because a similar osteoclastic response was not observed in the *Cox2*<sup>-/-</sup> mouse fracture callus (Figure 6). In addition, this large osteoclastic activity may be the causative factor involved in fracture destabilization by pin slippage, which was the major morbidity complicating these experiments (Figure 5).

[0052] Chondrocyte differentiation and persistence in the fracture callus appears to be altered by COX-2-selective NSAID treatment and by lack of *Cox2* (Figure 6). Cartilage that is evident in the 2-week fracture calluses of COX-2-selective NSAID treated rats either disappears or is dramatically reduced in the 3 and 4-week fracture calluses (Figure 3). In control rats, the fracture has bridged or is almost bridged by 4 weeks post-fracture as the cartilage present at early times had undergone normal endochondral ossification and is no longer evident. In contrast, cartilage within the indomethacin treated fracture callus does persist at 4 weeks post-fracture indicating a reduced rate of endochondral ossification. These observations suggest that the chondrocytes within the COX-2-selective NSAID treated rats deteriorated without forming a cartilage matrix in which endochondral ossification could occur. This phenomenon then leads to the development of fracture non-unions and mal-unions. Similar histological observations were seen in the *Cox2*<sup>-/-</sup> mouse fracture where chondrocytes present in the callus failed to form a mineralized matrix (Figure 6). In support of this hypothesis, teratocarcinoma chondrocytes developed from *Cox2*<sup>-/-</sup> embryonic stem cells were found to be deteriorating, hypotrophic, and undergoing apoptosis (Zhang et al. 2000). Additionally, the *Cox2*<sup>-/-</sup> mouse fracture

callus was much smaller than that from the *Cox1*<sup>-/-</sup> mouse suggesting that mesenchymal cell proliferation and migration into the *Cox2*<sup>-/-</sup> mouse fracture site is reduced or that the mesenchymal cells proportionately differentiate into fewer chondrocytes. Thus, COX-2 function is essential for normal progression of  
5 endochondral ossification during fracture healing.

[0053] There are several steps during endochondral ossification at which COX-2 could exert an essential regulatory function. Endochondral ossification is a complicated process that begins when chondrocytes mature to produce a cartilage matrix. Eventually the chondrocytes become hypertrophic and undergo apoptosis as  
10 the cartilage matrix matures and becomes calcified. Osteoclasts partially resorb the calcified cartilage with concurrent angiogenesis at the site of endochondral ossification. Osteoblasts proliferate and differentiate on the calcified cartilage and begin forming new bone. Remodeling of the new bone subsequently occurs to increase the mechanical properties of the bone and restore its normal architecture.  
15 Prostaglandins produced by COX-2 could be used to enhance osteoblast proliferation and differentiation (Kawaguchi et al. 1995). Prostaglandins may also be necessary to promote terminal differentiation of chondrocytes and formation of the cartilage matrix. Such an effect on chondrocytes could occur through a direct effect on differentiation or indirectly by preventing pre-mature apoptosis since inhibition of  
20 COX-2 function by COX-2-selective NSAIDs has been shown to induce apoptosis in cancer cell lines (Hsu et al. 2000). The potential effects of prostaglandins on osteoblasts and chondrocytes during endochondral ossification are not mutually exclusive. An additional possibility is that COX-2 dependent signaling occurs

between osteoblasts and chondrocytes to initiate and maintain endochondral ossification. Prostaglandins are also known to promote osteoclast activity, which may be essential for the normal endochondral ossification process. However, our histological observations indicate an exuberant osteoclast response at the fracture site

5 in the NSAID treated rats thus we do not favor this potential mechanism. Angiogenesis is also inhibited by COX-2-selective NSAIDs, at least in certain experimental models (Jones et al. 1999), and we failed to detect neovascularization of the *Cox2*<sup>-/-</sup> mouse fracture callus. Thus an additional possibility is that lack of angiogenesis precludes proper delivery of osteoclasts and osteoblasts to the cartilage

10 matrix interface for continued endochondral ossification.

[0054] Our observations suggest that COX-2-selective NSAIDs may be effective in reducing or preventing pathological heterotopic ossification. One particular genetic disease for which COX-2-selective NSAIDs may be efficacious is fibrodysplasia ossificans progressiva (FOP)(Cohen et al. 1993). Children afflicted

15 with FOP develop debilitating heterotopic bone through an endochondral ossification pathway that appears to initiate at sites of inflammation (Kaplan et al. 1993). Thus the COX-2-selective NSAIDs may be useful in reducing inflammation and stopping endochondral ossification at presumptive heterotopic ossification sites in children with FOP.

20 [0055] To determine if COX-2 functions in fracture healing, rats were treated with COX-2-selective non-steroidal anti-inflammatory drugs (NSAIDs) to stop COX-2-dependent prostaglandin production. Radiographic, histological, and mechanical testing demonstrated that fracture healing failed in rats treated with COX-2 selective

NSAIDs. Normal fracture healing also failed in mice homozygous for a null mutation in the COX-2 gene. These results demonstrate that COX-2 activity is necessary for normal fracture healing and confirms that the effects of COX-2-selective NSAIDs on fracture healing is due to inhibition of COX-2 activity and not from a drug side effect.

- 5 Furthermore, histological observations suggest that COX-2 is required for normal endochondral ossification during fracture healing. Since mice lacking *Cox2* form normal skeletons, our observations indicate that fetal bone development and fracture healing are different and that COX-2 function is specifically essential for fracture healing.

## 10 EXPERIMENTAL PROCEDURES

### *Animals, Drug Dosage, and Administration*

- [0056] A total of 253 male Sprague-Dawley rats ( $584 \pm 62\text{g}$ ) were fed a standard diet and kept caged separately in a constant temperature and humidity environment. All rats were 6-9 months old at the beginning of the experiment. Drugs
- 15 were administered daily by gavage beginning two days prior to fracture. Animals were randomly selected for each treatment group. The rats were gavaged with aqueous suspensions of indomethacin (1mg/kg), celecoxib (4mg/kg), or rofecoxib (3mg/kg). No drug (control) rats were not initially gavaged but later rats were gavaged with water and no difference was noted between the no drug rats that had
- 20 been gavaged and those that had not. No statistically significant differences were found in animal weight changes during the experiments.



[0057] Retired breeder female *Cox1*<sup>-/-</sup> (B6;129P2-*Ptgs1*<sup>tm1</sup>) and *Cox2*<sup>-/-</sup> (B6;129P2-*Ptgs2*<sup>tm1</sup>) mice were obtained from Taconic Farms. Closed femur fracture production was done using a method similar to that described below.

#### *Closed Femur Fracture Production*

5 [0058] The rats were anesthetized by intraperitoneal injection of ketamine (40 mg/kg) and xylazine (5 mg/kg). Under aseptic conditions, a medial parapatellar incision (0.4-0.5 cm) was made in the right hindlimb and the patella was dislocated laterally. The medullary canal was entered through the intercondylar notch and reamed with an 18-gauge needle. A 1.1 mm stainless steel pin (Small Parts Inc.,  
10 Miami Lakes, FL) was then inserted into the canal and secured in the proximal part of the greater trochanter by tamping. The distal portion of the pin was then cut flush with the femoral condyles and the patella dislocation was reduced. The soft tissue and skin were closed with 4-0 vicryl sutures. After closing, the diaphysis of the pinned femur was fractured by means of a three-point bending device as described by  
15 Bonnarens and Einhorn (Bonnarens and Einhorn 1984).

#### *Radiography*

[0059] Radiographs were made post-fracture to confirm the position and quality of each fracture and at sacrifice to determine the degree of healing. In addition several rats were selected randomly to produce serial radiographs (at least 2  
20 rats per treatment group). Radiographs were made of these rats weekly under anesthesia until the experimental endpoint (8 weeks). Radiographs of mice were also

made under anesthesia. All radiographs were made using a 43805N Faxitron (Hewlett-Packard, McMinnville, OR) and Kodak MinR-2000 mammography film.

### *Mechanical Testing*

[0060] Animals within each treatment group were sacrificed at 4, 6, and 8 weeks post-fracture by CO<sub>2</sub> asphyxiation. Animals with oblique, comminuted, or infected fractures were not used for mechanical testing. Both femora were removed and cleaned of all soft tissue leaving the fracture callus undisturbed and then immediately processed for mechanical testing. The samples were wrapped in saline soaked gauze to prevent dehydration between steps. Measurements of the femora were taken using digital calipers to determine femur length and external callus dimensions. The intramedullary pin was removed from the fractured femur and a 1mm-diameter stainless steel pin (~0.8cm length) was inserted at the proximal and distal end perpendicular to the long axis of the bone to prevent slipping in the potting material. The intact femur was also pinned as described above. The femoral ends were potted in 1-inch hexnuts using a low melt temperature metal (Wood's metal, Alfa Aesar, Ward Mill, MA). Once potted, the gage length (L) of each femur was measured. Torsional testing was conducted using a servohydraulic testing machine (MTS, Eden, Prairie, MN) with a 20Nm reaction torque cell (Interface, Scottsdale, AZ). The testing was carried out to failure at a rate of 2°/sec and a data recording rate of 20Hz. Both the fractured and intact femora were tested in internal rotation in proper anatomic orientation. The peak torque and angle at failure were calculated from the load-deformation curves. Internal fracture callus dimensions were measured

after mechanical testing. From the callus dimensions, the polar moment of inertia (J) was calculated based upon a hollow ellipse model (Bell et al. 1941; Engesaeter et al. 1978). The equations used to derive torsional rigidity, shear stress, shear modulus, and J were as follows (Popov 1968): (1) **Torsional Rigidity:**  $(T_{\max} \circ L) / \phi$  where  $T_{\max}$  is the peak torque value in Nmm, L is the gage length in mm, and  $\phi$  is the angle at failure in radians. (2) **Shear Stress:**  $(T_{\max} \circ R_{\max}) / J$  where  $R_{\max}$  is the largest radial dimension of the fracture callus in mm ( $a_o$ ) and J is the polar moment of inertia. (3) **Shear Modulus (G):**  $(T_{\max} \circ L) / J$ . (4) **Polar Moment of Inertia (J):**  $[\pi(ab^3 + a^3b - (a-t)(b-t)^3 - (a-t)^3(b-t))] / 4$  where a is  $[a_i + [(a_o - a_i) / 2]]$ ; b is  $[b_i + [(b_o - b_i) / 2]]$ ; t is the average bone thickness at the site of failure and is calculated as  $[(a_o - a_i) + (b_o - b_i)] / 2$  where  $a_o$  is the callus maximum outside radius,  $a_i$  is the maximum interior radius,  $b_o$  is the least outside radius, and  $b_i$  is the least interior radius in mm. Only torsional testing data for which the fractured and control femur tested without incident were used.

### *Histology*

[0061] Rats were sacrificed at 2, 3, 4, 6, and 8 weeks post-fracture by CO<sub>2</sub> asphyxiation. Both femora were resected and the stainless steel pin was removed from the medullary canal. The harvested femora were fixed in 10% buffered formalin and embedded in polymethylmethacrylate following standard histological techniques for calcified tissue. The samples were sectioned sagittally through the fracture callus using an Isomet diamond saw (Buehler Ltd., Lake Bluff, IL), mounted on plexiglass slides, and polished to a thickness of 100 $\mu$ m. The slides were then stained with van Gieson's picrofuchsin and Stevenel's blue in order to identify new bone growth and

cartilage formation (Maniopoulos et al. 1986). Mice femora were fixed, decalcified, paraffin embedded, sectioned, and stained with Masson's trichrome stain. The samples were viewed and photomicrographs were taken using an Olympus BH2-RFCA microscope or an Olympus SZ40 microscope.

5 ***Treatment with COX-2-selective NSAIDs leads to Fracture Non-Unions and Mal-Unions***

[0062] Femur fracture healing was followed by serial radiographic analysis of rats treated with celecoxib, rofecoxib, indomethacin, or gavaged daily with water (no drugs group). Radiographs were made immediately following fracture production and  
10 then every week till the end point of the experiments (8 weeks post-fracture). Representative results are shown in Figure 1.

[0063] We found that femur fracture healing proceeded normally in the no drug rats as expected. At 1 week post-fracture, formation of the hard callus could be detected radiographically but was more evident at 2 weeks (Figure 1A). By 4 weeks  
15 post-fracture, calcification of the soft callus was clearly evident indicating that endochondral ossification had occurred. Additionally, by 4 weeks post-fracture, the new bone formed during fracture repair had almost bridged the fracture gap. Bridging of the fracture and remodeling of the fracture callus were evident at 6 weeks post-fracture. Continued remodeling of the callus as well as remodeling of the original  
20 femoral cortical bone at the fracture site is clearly evident by 8 weeks post-fracture. These radiographic observations are typical of normal fracture healing.

[0064] Indomethacin treatment appeared to delay but not prevent fracture healing consistent with previous reports (Rø et al. 1976; Allen et al. 1980; Altman et

al. 1995). By 2 weeks post-fracture, an X-ray dense hard callus is clearly evident in the indomethacin treated rats (Figure 1B). However, bridging of the fracture gap did not appear to occur until 5-6 weeks post fracture as compared to approximately 4-5 weeks post-fracture in the untreated rats (compare Figures 1A and B). Bridging and remodeling were evident in the 8 week post-fracture radiographs of the indomethacin treated rats.

[0065] Celecoxib or rofecoxib treatment did not prevent formation of an X-ray dense hard callus as can be seen in the 2 and 4 week post-fracture radiographs (Figures 1C and D). However, the original fracture was still plainly evident in the celecoxib (Figure 1C) and rofecoxib (Figure 1D) treated rats even after 8 weeks. No rofecoxib treated rat was observed to have a normally bridged callus by radiography. However, non-unions, mal-unions, and unions of the fractured femurs were observed by radiography in the celecoxib treated rats. The mal-unions were typified by the radiograph seen in Figure 1C in which one cortex of the fracture callus was bridged but in which the original cortical bone ends of the fractured femur had not joined and the fracture was still clearly evident.

[0066] In addition to the serial radiographs made for certain rats, all animals in this study were examined radiographically immediately post-fracture and when euthanized. A random, blinded sample of the 8-week post-fracture radiographs were independently examined by 7 observers and scored as a union (1 point), mal-union (0.5 points), or non-union (0 points). Control rats had an average score of 0.71. In contrast, the indomethacin, celecoxib and rofecoxib treated rats had average scores of 0.54, 0.49, and 0.32, respectively. Despite the known difficulties associated with

judging fracture healing from radiographs (Nicholls et al. 1979; Panjabi et al. 1989), a statistical comparison between treatment groups showed that the no drug and rofecoxib groups were significantly different ( $P < 0.007$  using a Fisher's PLSD test at a 5% significance level).

- 5    [0067]        These observations clearly indicate that rofecoxib treatment inhibits fracture healing in rats leading to non-unions. It would also appear that at least by radiographic examination, celecoxib treatment negatively affects fracture healing to an extent similar to, if not worse than, indomethacin treatment.

10    *The Mechanical Properties of the Healing Femur Fracture Callus are Diminished by NSAID Treatment*

- [0068]        In conjunction with the radiographic analysis, torsional mechanical testing of fractured femurs was also performed. The fractured femur and contralateral control femur from rats at 4, 6, and 8 weeks post-fracture were tested to failure in torsion for each treatment group (no drugs, indomethacin, celecoxib, and rofecoxib).
- 15    The data from these tests is summarized in Figure 2 and Table 1. Peak torque is the maximum twisting force generated during torsional testing of the femur. Torsional rigidity is a measure of a structure's resistance to torque. Thus a bone with high torsional rigidity would fail after only a few degrees of rotation, but soft tissue would not reach its peak torque until after a large angular deflection. Maximum shear stress
- 20    is a measure of the ultimate shearing force withstood by the femur prior to failure and is a function of the applied torque and polar moment of inertia, which is dictated by callus geometry. Shear modulus measures the elastic resistance to deformation by a shearing stress for a given material and is constant for a given material.

[0069] We found in the no drug rats that the normalized peak torque (101%) and torsional rigidity (88%) of the fractured femur was restored by 8-weeks post-fracture as compared to the contralateral control femurs from each animal (Figure 2). However, the shear modulus (1.3 GPa) and normalized shear stress (48%) of the fractured femurs at 8 weeks post-fracture were still less than the contralateral control femurs (Figure 2, Table 1). This is the expected result because during fracture healing, the ultimate mechanical integrity of the fractured bone, that is peak torque, is maintained at a high level by increasing bone diameter via the fracture callus. Since the mechanical properties of the initially soft tissue within the callus and later the newly formed bone are much weaker than the mechanical properties of mature cortical bone; shear stress and shear modulus were, as expected, less than the contralateral control femurs. As the newly formed bone within the fracture callus matures by remodeling, the mechanical properties of the fractured bone increase. This is evident in our results as increases in shear stress and shear modulus with time (Figure 2). The high normalized torsional rigidity found for the fractured femurs in the no drug rats at 6 (89%) and 8 (88%) weeks post-fracture indicates that the fracture had been bridged by new bone as would be expected.

[0070] We also observed that all of the 6 and 8 week post-fracture no drug femurs and all the contralateral control femurs failed as predicted, mid-diaphyseal spiral fractures during the torsional mechanical testing.

[0071] Indomethacin treatment reduced the mechanical properties of the healing femur fractures at earlier time points (Figure 2). However by 8 weeks post-fracture, the normalized peak torque, torsional rigidity, and shear stress values

obtained from the indomethacin treated rats were not significantly different from the no drug rats. In contrast, at 6 weeks post-fracture, the normalized peak torque, torsional rigidity, and shear stress values (46, 34, and 15%, respectively) obtained from the indomethacin treated rats were less than the no drug rats at 6 weeks post-fracture (77, 89, and 36%, respectively). Pointedly, the significantly low torsional rigidity of the fractured femurs from the indomethacin treated rats at 6 weeks post-fracture indicates that the fracture had not been bridged by bone. Of the eight fractured femurs tested at 8 weeks post-fracture, 6 failed as unions, 1 failed as a mal-union, and 1 failed as a non-union (Table 2). These observations indicate that the non-selective NSAID, indomethacin, delays, but does not prevent fracture healing, which is consistent with previous studies and demonstrates the validity of our assay methods (Rø et al. 1976; Allen et al. 1980; Altman et al. 1995).

[0072] Rofecoxib treatment had a drastic effect on the mechanical properties of the fractured femurs. At 8 weeks post-fracture, for all values measured or derived, the mechanical properties of the fractured femurs from the rofecoxib treated rats were significantly less than the no drug rat fractured femurs (Figure 2, Table 1). At 8 weeks post-fracture, the fractured femurs of the rofecoxib treated rats had only obtained 50%, 31%, and 18% of peak torque, torsional rigidity, or maximum shear stress of the contralateral unfractured femurs, respectively. The low torsional rigidity and shear modulus (0.5 GPa) values obtained from the fractured femurs of the rofecoxib treated rats are consistent with healing failure and the formation of non-unions. In addition, whereas all of the contralateral control femurs from the rofecoxib treated rats failed as mid-diaphyseal spiral fractures, 4 of the 5 fractured femurs at 8



weeks post-fracture failed as non-unions and the other failed as a malunion (Table 2). These observations demonstrate that, at the dose and treatment regime employed, the COX-2-selective NSAID rofecoxib stops fracture healing.

[0073] Unlike rofecoxib treatment, no significant differences were found in  
5 the mechanical properties of the healing fractured femurs from the celecoxib treated rats as compared to no drug rats. Despite the overall similarities in the mechanical values obtained between no drug rats and celecoxib treated rats, 3 of 6 fractured femurs from the celecoxib treated rats at 8 weeks post-fracture failed as non-unions during the mechanical testing procedure and the other 3 failed as mal-unions (Table  
10 2). The relatively low normalized torsional rigidity (50%) and shear stress (22%) found for fractured femurs from the celecoxib treated rats at 6 weeks post-fracture indicates that the fracture site had not been bridged with bone (Figure 2). Even though not statistically different from the no drug rat fractured femurs, the data obtained from the celecoxib treated rat fractured femurs parallels closely the patterns  
15 obtained from the femurs of the indomethacin treated rats. Together these observations suggest that, at the celecoxib dose and treatment regime used, fracture healing is delayed and to a lesser extent than that found for the rofecoxib treatment regime, inhibited.

[0074] A  $\chi^2$  analysis was performed on visual inspection data obtained from  
20 the 8 week post-fracture femurs following mechanical testing (Table 2). The fractured femurs were considered to have failed as (a) unions if a spiral fracture developed through the diaphysis of the femur, (b) non-unions if the femur failed completely along the original fracture site, and (c) mal-unions if some new bone

bridging of the fracture site was evident but that the femur still failed primarily along the original fracture site. The data from the no drug, celecoxib, and rofecoxib treatment groups were compared to that from the indomethacin treatment group. Our analysis indicates that no statistical difference exists between the no drug and the  
5 indomethacin treatment groups but that the celecoxib and rofecoxib treatment groups are significantly different from the indomethacin treatment group. Again, these observations strongly indicate that inhibition of COX-2 dramatically inhibits fracture healing.

[0075] No significant differences in the mechanical properties of the  
10 contralateral femurs were found between treatment groups. This indicates that the experimental treatment regimes did not alter the intrinsic properties of the rat bone by enhanced bone resorption or deposition at least for the time frame examined.

***COX-2-selective NSAID Treatment Alters Cartilage Formation during Fracture Healing***

15 [0076] The radiographic and torsional mechanic testing analyses of the COX-2-selective NSAID treated rats demonstrated that these drugs dramatically inhibit fracture healing. However, hard callus formation appeared not to be impaired in the COX-2-selective NSAID treated rats. This suggests that COX-2-selective NSAIDs impair fracture healing in the soft callus where endochondral ossification occurs.  
20 Since radiography cannot assess the early stages of endochondral ossification in the soft callus, we undertook a histological analysis of fracture healing in the drug treated rats.

[0077] At 2 weeks post-fracture, gross abnormalities were present in the histology of the healing femur fractures of the indomethacin, celecoxib, or rofecoxib treated rats as compared to an untreated control rat (Figure 3). In all four experimental groups, significant periosteal intramembraneous ossification was evident at the fracture site as expected from our radiographic data. Endochondral ossification also appeared to be proceeding normally in the no drug rat specimens. In contrast, the histological specimens from the indomethacin and COX-2-selective NSAID treated rats had abnormally formed cartilage elements within the callus. The positional extent of new bone formed in the callus of the NSAID treated rats also appeared to be abnormal in that it did not fully extend to the ends of the fractured bone. In the no drug rats, new bone in the callus extends to the very ends of the cortical bone fracture site. This is not so in the NSAID treated rats where this region of the callus is generally occupied by cartilage.

[0078] NSAID treatment grossly altered fracture callus morphology at 3 and 4 weeks post-fracture (Figure 3). Fracture healing proceeded normally in the no drug rats with near bridging of the callus apparent by 4 weeks post-fracture in concurrence with our radiographic data (Figure 1). However, healing was clearly delayed in the NSAID treated rats. The fracture calluses of the indomethacin treated rats were not bridged at 4 weeks but still appeared to be undergoing endochondral ossification based upon the presence of cartilage within the soft callus at 3 and 4 weeks post-fracture. In contrast, little or no cartilage was evident in the fracture calluses of the celecoxib or rofecoxib treated rats at 3 and 4 weeks post-fracture indicating that endochondral ossification had ceased. Additionally massive resorption of the woven

bone in the hard callus of the NSAID treated rats appeared to leave a shell-like callus on the ends of the fractured bone.

[0079] Celecoxib and rofecoxib treatment often caused a massive bone resorption event at the distal ends of the fracture bones leaving what appear to be indentations into the hard callus (Figure 3 and 4). The magnitude of this bone resorption phase is indicated by the large number of osteoclasts that were found on the femur periosteal surface at the distal ends of the fractured bone near the apparent indentation (Figure 4).

[0080] At 6 and 8 weeks post-fracture, the fractured femurs of the no drug rats appeared to be healing normally with active remodeling of the cortical bone ends and fracture callus. Fractured femurs from indomethacin treated rats also appeared to be healing at 6 and 8 weeks post-fracture with evident bridging and active remodeling. In contrast, no further healing was evident in the celecoxib or rofecoxib treated rat fractured femurs. The callus in the COX-2-selective NSAID treated rats was smaller at 6 and 8 weeks post-fracture but the fracture gap was still clearly evident and often filled with fibrous tissue. These observations are consistent with our radiographic and torsional mechanical testing data demonstrating that celecoxib and rofecoxib inhibit fracture healing.

#### *Complications Associated with Use of COX-2-Selective NSAIDs*

[0081] As can be seen in Figure 5, pin slippage was a severe complication with as many as 30% of the rofecoxib treated rats having to be euthanized prior to the endpoint. Pin slippage is dislodgement of the intramedullary stainless steel rod used to stabilize the fracture and permit the rat to weight-bear on the fractured femur.

Once fracture stability was lost, the rat was euthanized since the rat could no longer weight-bear on the femur and since the callus would be re-injured and thus alter healing. The etiology of the pin slippage is unknown. Animals with these complications were excluded. As such, final data may be skewed in favor of rats that had healed. Other complications included anesthesia death during weekly radiographs and infections that excluded specimens from further analysis. It was found using a  $\chi^2$  analysis to compare each experimental group value to the no drug rat value that the pin slippage rate was significant for all NSAID treatment groups ( $P < 0.0001$ ) and that the infection rate for the rofecoxib treated rats was also significant ( $P < 0.0001$ ).

#### *Normal Fracture Healing Fails in Cox2 Null Mice*

[0082] Our data clearly demonstrate that COX-2-selective NSAIDs are detrimental to fracture healing. Unfortunately, these observations do not distinguish between a specific effect on COX-2 and a non-specific effect of the NSAIDs on fracture healing. Therefore to specifically address whether fracture healing requires *Cox1* or *Cox2* gene function, femur fracture healing was assessed in *Cox1* and *Cox2* knock-out mice (Langenbach et al. 1995; Morham et al. 1995). Using a modified method, closed femur fractures were produced in three female *Cox1*<sup>-/-</sup> (*Cox1* knock-out) and three female *Cox2*<sup>-/-</sup> (*Cox2* knock-out) mice. The animals were examined radiographically immediately post-fracture and then at 7, 10, 14, 21, 28, and 42 days post-fracture. Fracture healing appeared to proceed normally in the *Cox1*<sup>-/-</sup> mice relative to our previous observations in outbred and inbred strains of mice (Manigrasso and O'Connor, unpublished). In contrast, only a slight periosteal hard

callus was detected in any of the *Cox2*<sup>-/-</sup> mice, which is indicative of healing failure. As can be seen in Figure 6, the apparent difference in fracture callus size was most obvious at 2 weeks post-fracture when the *Cox1*<sup>-/-</sup> mice had formed a large fracture callus but little or no callus was evident in the *Cox2*<sup>-/-</sup> mice.

5 [0083] One mouse of each genotype was euthanized at 2 weeks post-fracture and the fractured femur examined histologically (Figure 6). New bone and differentiating chondrocytes were abundant within the *Cox1*<sup>-/-</sup> callus indicating that COX-1 activity is not essential for fracture healing. In contrast, there was a plainly evident lack of new bone formation in the *Cox2*<sup>-/-</sup> fracture callus with only some  
10 apparent intramembraneous bone formation occurring at the edges of the callus (hard callus). Chondrocytes at different stages of differentiation were observed throughout the *Cox2*<sup>-/-</sup> soft fracture callus. However, the *Cox2*<sup>-/-</sup> chondrocytes failed to form a mineralized matrix as evident by the radiolucency and histological appearance of the soft callus. The amount of endochondral ossification at the hard callus-soft callus  
15 boundary appeared greatly reduced in the *Cox2*<sup>-/-</sup> specimen. In addition, neovascularization of the *Cox2*<sup>-/-</sup> fracture callus was not observed. These observations clearly indicate that normal fracture healing and endochondral ossification are stopped or dramatically reduced in the *Cox2*<sup>-/-</sup> mice and confirms our observations made in the COX-2-selective NSAID treated rats.

20 [0084] Our data indicate that COX-2 function is essential for fracture healing. In contrast, adult mice homozygous for targeted mutation of *Cox2* appear to have normal skeletons. Together these observations demonstrate that fetal osteogenesis and fracture healing, though similar in many ways, are different and are probably

initiated and maintained through different molecular mechanisms. *Cox2* is the first gene to be identified that is specifically essential for fracture healing but not fetal osteogenesis. Targeted mutation of other mouse genes involved in prostaglandin synthesis and signaling should enable further analysis of the prostaglandin pathway(s) involved in fracture healing and skeletal biology in general.

[0085] One skilled in the art will readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned as well as those inherent therein. The cyclooxygenase vectors along with the methods, procedures and treatments described herein are presently representative of preferred embodiments and are exemplary and not intended as limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art which are encompassed within the spirit of the invention or defined by this scope with the claims.

[0086] It will be readily apparent to one skilled in the art that varying substitutions and modifications may be made to the invention disclosed herein within departing from the scope and spirit of the invention.

[0087] All patents and publications referenced herein are incorporated by reference to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference. The following references are likewise incorporated by reference in order to more fully describe the state of the art.

## REFERENCES

- [0088] Allen, H.L., A. Wase, and W.T. Bear. 1980. Indomethacin and aspirin: effect of nonsteroidal anti-inflammatory agents on the rate of fracture repair in the rat. *Acta. Orthop. Scand.* **51**: 595-600.
- 5 [0089] Altman, R.D., L.L. Latta, R. Keer, K. Renfree, F.J. Hornicek, and K. Banovac. 1995. Effect of nonsteroidal antiinflammatory drugs on fracture healing a laboratory study in rats. *J. Orthop. Trauma* **9**: 392-400.
- [0090] Bell, G.H., D.P. Cuthbertson, and J. Orr. 1941. Strength and size of bone in relation to calcium intake. *Journal of Physiology* **100**: 299-317.
- 10 [0091] Bolander, M.E. 1992. Regulation of fracture repair by growth factors. *Proc. Soc. Exp. Biol. Med.* **200**: 165-170.
- [0092] Bonnarens, F. and T.A. Einhorn. 1984. Production of a standard closed fracture in laboratory animal bone. *J. Orthop. Res.* **2**: 97-101.
- [0093] Brighton, C.T., J.L. Schaffer, D.B. Shapiro, J.J.S. Tang, and C.C. Clark. 1991. Proliferation and macromolecular synthesis by rat calvarial bone cells grown in various oxygen tensions. *J. Orthop. Res.* **9**: 847-854.
- 15 [0094] Cohen, R.B., G.V. Hahn, J.A. Tabas, J. Peeper, C.L. Levitz, A. Sando, N. Sando, M. Zasloff, and F.S. Kaplan. 1993. The natural history of heterotopic ossification in patients who have fibrodysplasia ossificans progressiva. *J. Bone and Joint Surg.* **75-A**: 215-219.
- 20 [0095] Davies, N.M., A.J. McLachlan, R.O. Day, and K.M. Williams. 2000. Clinical pharmacokinetics and pharmacodynamics of celecoxib: a selective cyclo-oxygenase-2 inhibitor. *Clinical Pharmacokinetics* **38**: 225-242.



- [0096] Dekel, S., G. Lenthall, and M.J.O. Francis. 1981. Release of prostaglandins from bone and muscle after tibial fracture. *J. Bone and Joint Surg.* 63-B: 185-189.
- [0097] Depré, M., E. Ehrich, A. van Hecken, I. De Lepeleire, A. Dallob, P. Wong, A. Porras, B.J. Gertz, and P.J. De Schepper. 2000. Pharmacokinetics, COX-2 specificity, and tolerability of supratherapeutic doses of rofecoxib in humans. *European Journal of Pharmacology* 56: 167-174.
- [0098] Einhorn, T.A. 1998. The cell and molecular biology of fracture healing. *Clin Orthop*: S7-21.
- 10 [0099] Engesaeter, L.B., A. Ekeland, and N. Langeland. 1978. Methods for testing the mechanical properties of the rat femur. *Acta. Orthop. Scand.* 49: 512-518.
- [0100] Ferguson, C., E. Alpern, T. Miclau, and J.A. Helms. 1999. Does adult fracture repair recapitulate embryonic skeletal formation? *Mech. Dev.* 87: 57-66.
- [0101] Feyen, J.H., G. van der Wilt, P. Moonen, A. Di Bon, and P.J. Nijweide. 15 1984. Stimulation of arachidonic acid metabolism in primary cultures of osteoblast-like cells by hormones and drugs. *Prostaglandins* 28: 769-781.
- [0102] Halpin, R.A., L.A. Geer, K.E. Zhang, T.M. Marks, D.C. Dean, A.N. Jones, D. Melillo, G. Doss, and K.P. Vyas. 2000. The absorption, distribution, metabolism and excretion of rofecoxib, a potent and selective cyclooxygenase-2 20 inhibitor, in rats and dogs. *Drug Metabolism and Disposition* 28: 1244-1254.
- [0103] Hsu, A.-L., T.-T. Ching, D.-S. Wang, X. Song, V.M. Rangnekar, and C.-S. Chen. 2000. The cyclooxygenase-2 inhibitor celecoxib induces apoptosis by

blocking Akt activation in human prostate cancer cells independently of Bcl-2. *J. Biol. Chem.* **275**: 11397-11403.

- [0104] Jones, M.K., H. Wang, B.M. Peskar, E. Levin, R.M. Itani, I.J. Sarfeh, and A.S. Tarnawski. 1999. Inhibition of angiogenesis by nonsteroidal anti-inflammatory drugs: insight into the mechanisms and implications for cancer growth and ulcer healing. *Nat. Med.* **5**: 1418-1423.
- [0105] Kaplan, F.S., J.A. Tabas, F.H. Gannon, G. Finkel, G.V. Hahn, and M.A. Zasloff. 1993. The histopathology of fibrodysplasia ossificans progressiva: an endochondral process. *J. Bone and Joint Surg.* **75-A**: 220-230.
- 10 [0106] Kawaguchi, H., C.C. Pilbeam, J.R. Harrison, and L.G. Raisz. 1995. The role of prostaglandins in the regulation of bone metabolism. *Clin. Orth. Rel. Res.* **313**: 36-46.
- [0107] Klein, D.C. and L.G. Raisz. 1970. Prostaglandins: stimulation of bone resorption in tissue culture. *Endocrinol.* **86**: 1436-1440.
- 15 [0108] Klein-Nulend, J., E.H. Burger, C.M. Semeins, L.G. Raisz, and C.C. Pilbeam. 1997. Pulsating fluid flow stimulates prostaglandin release and inducible prostaglandin G/H synthase mRNA expression in primary mouse bone cells. *J. Bone Miner. Res.* **12**: 45-51.
- [0109] Langenbach, R., S.G. Morham, H.F. Tiano, C.D. Loftin, B.I. Ghanayem, P.C. Chulada, J.F. Mahler, C.A. Lee, E.H. Goulding, K.D. Kluckman, H.S. Kim, and O. Smithies. 1995. Prostaglandin synthase 1 gene disruption in mice reduces arachidonic acid-induced inflammation and indomethacin-induced gastric ulceration. *Cell* **83**: 483-492.
- 20

- [0110] Maniatopoulos, C., A. Rodriguez, D.A. Deporter, and A.H. Melcher. 1986. An improved method for preparing histological sections of metallic implants. *International Journal of Oral and Maxillofacial Implants* **1**: 31-37.
- [0111] Masferrer, J.L., B.S. Zweifel, P.T. Manning, S.D. Hauser, K.M. Leahy, 5 W.G. Smith, P.C. Isakson, and K. Seibert. 1994. Selective inhibition of inducible cyclooxygenase 2 *in vivo* is antiinflammatory and nonulcerogenic. *Proc. Natl. Acad. Sci. USA* **91**: 3228-3232.
- [0112] Moore, K.D., K. Goss, and J.O. Anglen. 1998. Indomethacin *versus* radiation therapy for prophylaxis against heterotopic ossification in acetabular 10 fractures. *J. Bone and Joint Surg.* **80-B**: 259-263.
- [0113] Morham, S.G., R. Langenbach, C.D. Loftin, H.F. Tiano, N. Vouloumanos, J.C. Jennette, J.F. Mahler, K.D. Kluckman, A. Ledford, C.A. Lee, and O. Smithies. 1995. Prostaglandin synthase 2 gene disruption causes severe renal pathology in the mouse. *Cell* **83**: 473-482.
- 15 [0114] Muscará, M.N., W. McKnight, S. Asfaha, and J.L. Wallace. 2000. Wound collagen deposition in rats: effects of an NO-NSAID and a selective COX-2 inhibitor. *British Journal of Pharmacology* **129**: 681-686.
- [0115] Needleman, P., J. Turk, B.A. Jakschik, A.R. Morrison, and J.B. Lefkowitz. 1986. Arachidonic acid metabolism. *Ann. Rev. Biochem.* **55**: 69-102.
- 20 [0116] Nicholls, P.J., E. Berg, F.E. Bliven, Jr., and J.M. Kling. 1979. X-ray diagnosis of healing fractures in rabbits. *Clin. Orth. Rel. Res.* **142**: 234-236.

- [0117] O'Banion, M.K., V.D. Winn, and D.A. Young. 1992. cDNA cloning and functional activity of a glucocorticoid-regulated inflammatory cyclooxygenase. *Proc. Natl. Acad. Sci. USA* **89**: 4888-4892.
- [0118] Okada, Y., J.A. Lorenzo, A.M. Freeman, M. Tomita, S.G. Morham,  
5 L.G. Raisz, and C.C. Pilbeam. 2000. Prostaglandin G/H synthase-2 is required for maximal formation of osteoclast-like cells in culture. *J. Clin. Invest.* **105**: 823-832.
- [0119] O'Sullivan, M.G., F.H. Chilton, E.M. Huggins, Jr., and C.E. McCall. 1992a. Lipopolysaccharide priming of alveolar macrophages for enhanced synthesis of prostanoids involves induction of a novel prostaglandin H synthase. *J. Biol. Chem.*  
10 **267**: 14547-14550.
- [0120] O'Sullivan, M.G., E.M. Huggins, Jr., E.A. Meade, D.L. DeWitt, and C.E. McCall. 1992b. Lipopolysaccharide induces prostaglandin H synthase-2 in alveolar macrophages. *Biochem. Biophys. Res. Comm.* **187**: 1123-1127.
- [0121] Panjabi, M.M., R.W. Lindsey, S.D. Walter, and A.A. White, 3rd. 1989.  
15 The clinician's ability to evaluate the strength of healing fractures from plain radiographs. *J. Orthop. Trauma* **3**: 29-32.
- [0122] Paulson, S.K., J.Y. Zhang, A.P. Breau, J.D. Hribar, N.W.K. Liu, S.M. Jessen, Y.M. Lawal, J.N. Cogburn, C.J. Gresk, C.S. Markos, T.J. Maziasz, G.L. Schoenhard, and E.G. Burton. 2000. Pharmacokinetics, tissue distribution,  
20 metabolism, and excretion of celecoxib in rats. *Drug Metabolism and Disposition* **28**: 514-521.
- [0123] Popov, E.P. 1968. *Introduction to mechanics of solids*. Prentice-Hall, Inc., Englewood Cliffs, New Jersey.

- [0124] Pritchett, J.W. 1995. Ketorolac prophylaxis against heterotopic ossification after hip replacement. *Clin. Orth. Rel. Res.* 314: 162-165.
- [0125] Raskin, J.B. 1999. Gastrointestinal effects of nonsteroidal anti-inflammatory therapy. *Am. J. Med.* 106(5B): 3S-12S.
- 5 [0126] Riendeau, D., M.D. Percival, C. Brideau, S. Charleson, D. Dube, D. Ethier, J.-P. Falgueyret, R.W. Friesen, R. Gordon, G. Greig, J. Guay, J. Mancini, M. Ouellet, E. Wong, L. Xu, S. Boyce, D. Visco, Y. Girard, P. Prasit, R. Zamboni, I.W. Rodger, M. Gresser, A.W. Ford-Hutchinson, R.N. Young, and C.-C. Chan. 2001. Etoricoxib (MK-0663): preclinical profile and comparison with other agents that  
10 selectively inhibit cyclooxygenase-2. *Journal of Pharmacology and Experimental Therapeutics* 296: 558-566.
- [0127] Rø, J., N. Langeland, and J. Sander. 1978. Effect of indomethacin on collagen metabolism of rat fracture callus *in vitro*. *Acta. Orthop. Scand.* 49: 323-328.
- [0128] Rø, J., E. Sudmann, and P.F. Marton. 1976. Effect of indomethacin on  
15 fracture healing in rats. *Acta. Orthop. Scand.* 47: 588-599.
- [0129] Rossi, A., P. Kapahi, G. Natoli, T. Takahashi, Y. Chen, M. Karin, and M.G. Santoro. 2000. Anti-inflammatory cyclopentenone prostaglandins are direct inhibitors of IκB kinase. *Nature* 403: 103-108.
- [0130] Shigeta, J.-I., S. Takahashi, and S. Okabe. 1998. Role of  
20 cyclooxygenase-2 in the healing of gastric ulcers in rats. *Journal of Pharmacology and Experimental Therapeutics* 286: 1383-1390.
- [0131] Topper, J.N., J. Cai, D. Falb, and M.A. Gimbrone, Jr. 1996. Identification of vascular endothelial genes differentially responsive to fluid

mechanical stimuli: cyclooxygenase-2, manganese superoxide dismutase, and endothelial cell nitric oxide synthase are selectively up-regulated by steady laminar shear stress. *Proc. Natl. Acad. Sci. USA* **93**: 10417-10422.

[0132] Vane, J.R., Y.S. Bakhle, and R.M. Botting. 1998. Cyclooxygenase 1  
5 and 2. *Annual Review of Pharmacology and Toxicology* **38**: 97-120.

[0133] Vortkamp, A., S. Pathi, G.M. Peretti, E.M. Caruso, D.J. Zaleske, and C.J. Tabin. 1998. Recapitulation of signals regulating embryonic bone formation during postnatal growth and fracture repair. *Mech. Dev.* **71**: 65-76.

[0134] Wadleigh, D.J. and H.R. Herschman. 1999. Transcriptional regulation  
10 of the cyclooxygenase-2 gene by diverse ligands in murine osteoblasts. *Biochem. Biophys. Res. Comm.* **264**: 865-870.

[0135] Wallace, J.L., A. Bak, W. McKnight, S. Asfaha, K.A. Sharkey, and W.K. MacNaughton. 1998. Cyclooxygenase 1 contributes to inflammatory responses in rats and mice: implications for gastrointestinal toxicity. *Gastroenterology* **115**: 101-  
15 109.

[0136] Warner, T.D., F. Giuliano, I. Vojnovic, A. Bukasa, J.A. Mitchell, and J.R. Vane. 1999. Nonsteroid drug selectivities for cyclo-oxygenase-1 rather than cyclo-oxygenase-2 are associated with human gastrointestinal toxicity: a full *in vitro* analysis. *Proc. Natl. Acad. Sci. USA* **96**: 7563-7568.

20 [0137] Whelton, A. 1999. Nephrotoxicity of nonsteroidal anti-inflammatory drugs: physiological foundations and clinical implications. *Am. J. Med.* **106(5B)**: 13S-24S.

[0138] White, A.A., III, M.M. Panjabi, and W.O. Southwick. 1977. The four biomechanical stages of fracture repair. *J. Bone and Joint Surg.* **59-A**: 188-192.

[0139] Xie, W., J.G. Chipman, D.L. Robertson, R.L. Erikson, and D.L. Simmons. 1991. Expression of a mitogen-responsive gene encoding prostaglandin synthase is regulated by mRNA splicing. *Proc. Natl. Acad. Sci. USA* **88**: 2692-2696.

[0140] Zhang, X., S.G. Morham, R. Langenbach, R.B. Baggs, and D.A. Young. 2000. Lack of cyclooxygenase-2 inhibits growth of teratocarcinomas in mice. *Exp. Cell Res.* **254**: 232-240.

[00100]

Table 1. Torsional Mechanical Testing Data

All Contralateral Control Femurs	Fractured Femur Data (not normalized to contralateral control femur)											
	No Drug			Indomethacin			Celecoxib			Rofecoxib		
	4 wks	6 wks	8 wks	4 wks	6 wks	8 wks	4 wks	6 wks	8 wks	4 wks	6 wks	8 wks
Peak Torque (Tmax) in Nmm												
Mean	716	452	430	643	417	357	469	302	567	620	250	338
CV	28	21	29	28	52	37	56	44	34	49	53	36
Significance	—	—	—	—	0.69	0.36	0.16	0.03	0.15	0.88	0.01	0.01
Torsional Rigidity [(Tmax • L)/φ]												
Mean	83,050	27,200	66,068	56,506	21,913	28,658	57,630	13,866	43,189	59,256	12,338	21,231
CV	35	48	17	20	46	57	55	53	77	22	59	51
Significance	—	—	—	—	0.43	<0.01	0.93	0.06	0.11	0.69	0.04	<0.01
Shear Stress [(Tmax • φ)/J]												
mean	145	33	35	56	25	22	47	25	44	67	15	24
CV	38	42	34	27	85	47	66	73	73	41	58	41
Significance	—	—	—	—	0.42	0.08	0.48	0.37	0.47	0.40	0.03	0.13
Shear Modulus (GPa)												
Mean	6.9	0.4	1.5	1.3	0.3	0.4	1.8	0.3	0.9	1.7	0.2	0.3
CV	54	58	71	38	78	73	99	77	116	27	49	56
Significance	—	—	—	—	0.33	0.08	0.46	0.26	0.40	0.16	0.04	0.07
Sample Size	81	6	5	7	8	7	8	8	8	6	6	7
												5

CV: Coefficient of Variation (standard deviation as a percentage).



Significance: Comparisons were made using a standard two-tailed T-test between the no drug fractured femur values and the experimental fractured femur values within a time point. These comparisons were not made between data normalized with the contralateral control femur as shown in Figure 3.

Table 2. Frequency of Unions, Mal-Unions, and Non-Unions in the Mechanically Tested Fractured Femurs at 8 weeks Post-Fracture

	Union (Stage IV)*	Mal-Union (Stage III)	Non-Union (Stage I/II)	$\chi^2$ P-value**
No Drugs	7	0	0	0.338
Indomethacin	6	1	1	—
Celecoxib	0	3	3	<0.001
Rofecoxib	0	1	4	<0.001

\* Classification scheme as described by White et al. (1977)

\*\*Comparisons were made to the indomethacin treatment group data.

## CLAIMS

What is claimed is:

- 5           1.       A vector for use in enhancing wound healing comprising a promoter linked to a cyclooxygenase expression cassette.
2.       The vector of claim 1, wherein the promoter is constitutively induced.
3.       The vector of claim 2, wherein the promoter is a cytomegalovirus promoter.
- 10          4.       The vector of claim 1, wherein the cyclooxygenase expression cassette encodes a COX-2 gene product.
5.       A method for enhancing wound healing comprising the step of delivering the vector of claim 1 to the location of the wound; wherein the cyclooxygenase expression cassette is expressed, thereby enhancing wound healing.
- 15          6.       The method of claim 5, wherein the vector is a adenoviral vector, adeno-associated viral vector, a recombination-defective retrovirus or a plasmid.
7.       The method of claim 5, wherein the vector is delivered by injection, electroporation, or inhalation of an aerosol.
8.       The method of claim 5, wherein the vector is in a saline solution, 20 encapsulated in liposomes, in a polymer solution, in a gel, or lyophilized.
9.       The method of claim 5, wherein the promoter is cytomegalovirus.
10.       The method of claim 5, wherein the cyclooxygenase expression cassette encodes for a COX-2 gene product.

11. The method of claim 5, wherein the wound is a bone fracture or a skin wound.

12. A method for enhancing wound healing following orthopaedic procedures comprising the step of administering the vector of claim 1 to the location  
5 of the procedure; wherein the cyclooxygenase expression cassette encodes for COX-2 gene product and wherein the cyclooxygenase expression cassette is expressed thereby enhancing wound healing.

13. A method for treating pathological heterotopic ossification conditions comprising the steps of:  
10 identifying locations of heterotopic ossification in a patient with a heterotopic ossification condition, and  
administering COX-2 selective NSAIDs to the patient.

14. The method of claim 13, wherein the heterotopic ossification condition is fibrodysplasia ossificans progressiva, occurs following a hip replacement procedure  
15 or after an acetabular fracture.

15. A method for inhibiting wound healing comprising the step of administering an effective amount of NSAIDs to the location of the wound, wherein the NSAIDs inhibit normal wound healing.

16. The method of claim 15, wherein the NSAIDs are COX-2 specific  
20 NSAIDs.

17. A method for treating osteoporosis, OI and brittle bone conditions comprising the step of administering the vector of claim 1, wherein expression of the vector enhances wound healing and bone formation.

18. A composition for use in wound healing comprising a formulated  
.5 cyclooxygenase protein.

19. The composition of claim 18, further comprising a pharmaceutically acceptable carrier.

20. The composition of claim 18, wherein the cyclooxygenase protein is COX-1, COX-2 or a combination of the two.

10 21. A method for treating wounds comprising the step administering the composition of claim 18 to a patient with a wound.

22. The method of claim 21, wherein the composition is administered topically, orally, intravenously, nasally or locally.

23. A method of enhancing wound healing, wherein prostaglandin  
15 production is stimulated in at least one osteoblast located at or near a wound.

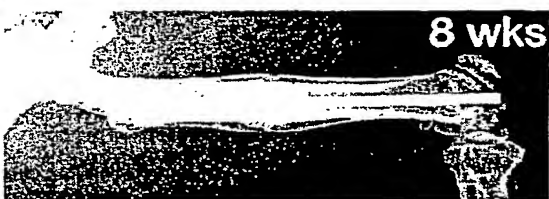
24. A method of enhancing wound healing comprising the step of delivering stable prostaglandins to the location of a wound.

25. The method of claim 24, wherein the stable prostaglandins are delivered topically, orally, intravenously, nasally or locally.

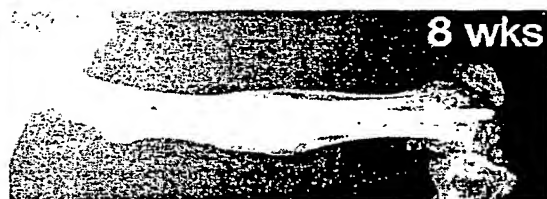
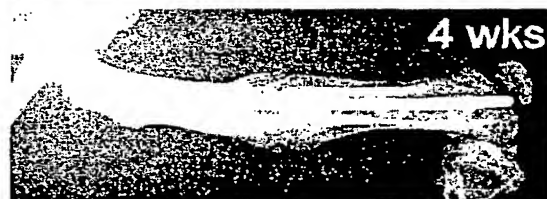
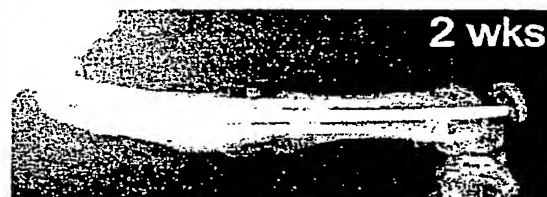
20

**Figure 1. Radiographic Analysis of Fracture Healing in COX2-Selective NSAID Treated Rats**

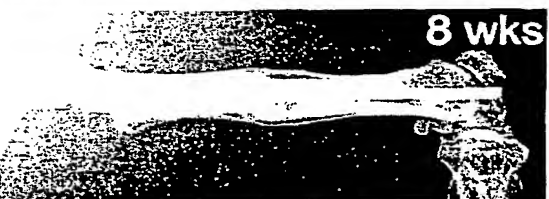
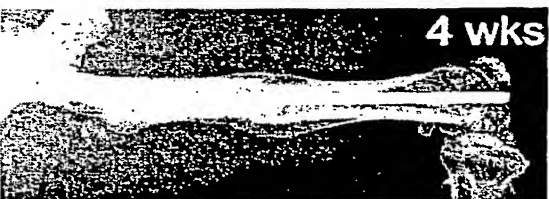
**A. Control**



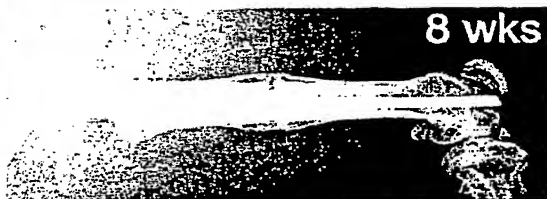
**B. Indomethacin**



**C. Celecoxib**

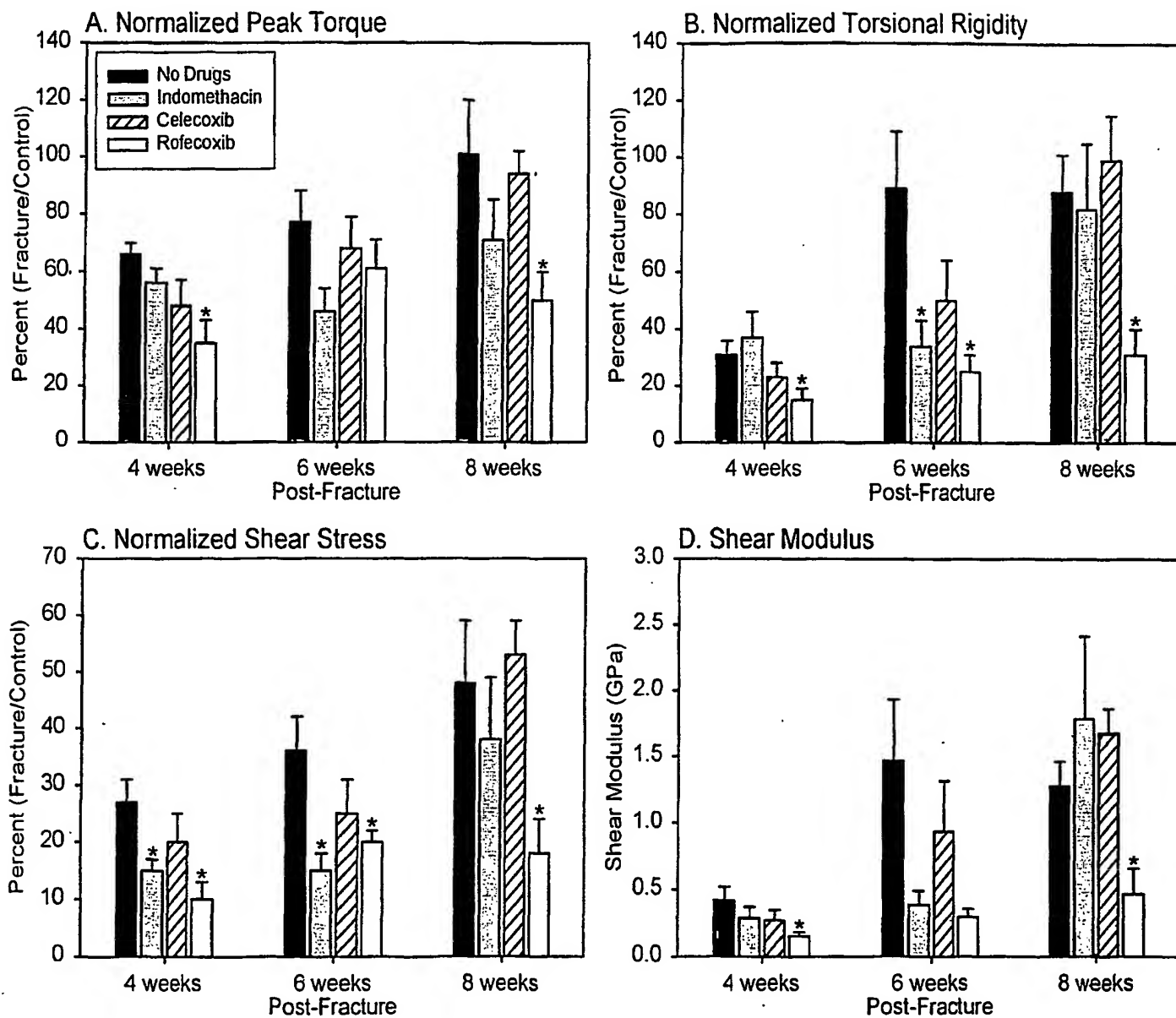


**D. Rofecoxib**

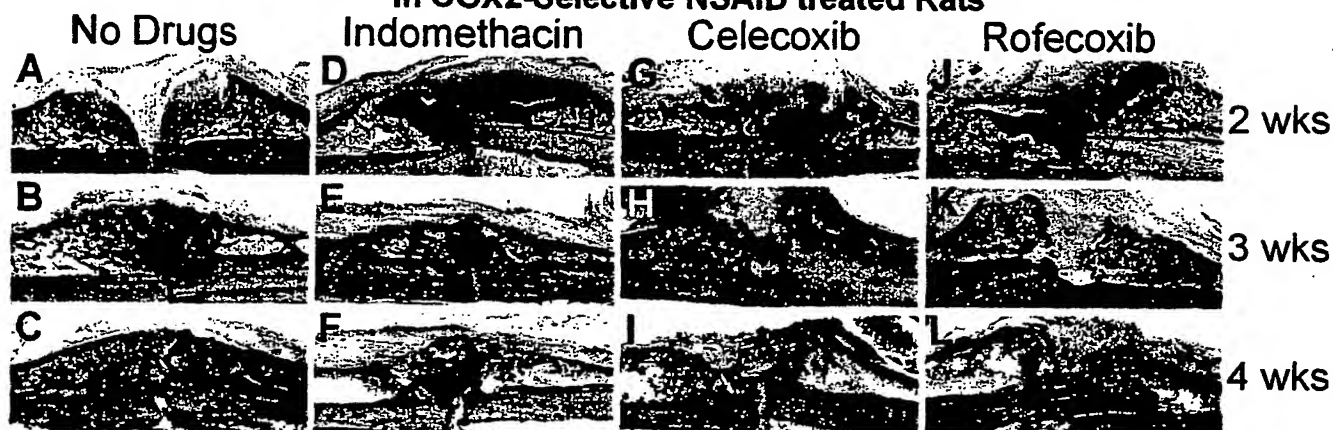


Radiographs were made using an HP Faxitron and Kodak MINR2000 film. Treatment groups and post-fracture time points are indicated.

Figure 2. Rofecoxib Treatment Reduces Fracture Callus Mechanical Properties (\* indicates  $P < 0.05$  as per t-test)



**Figure 3. Endochondral Ossification Fails during Fracture Healing in COX2-Selective NSAID treated Rats**



Fractured femurs were fixed and embedded in PMMA. The specimens were sectioned sagittally through the fracture callus and stained with van Gieson's picrofuchsin and Stevenel's bue. Drug treatment and times post-fracture are indicated. Note the lack of chondrocytes and cartilage in the 3 and 4 week post-fracture celecoxib and rofecoxib treated specimens indicative of failed endochondral ossification.



**Figure 4. Osteoclast activity (4-weeks post-fracture) on the cortical bone next to the fracture site in rofecoxib treated rats.**

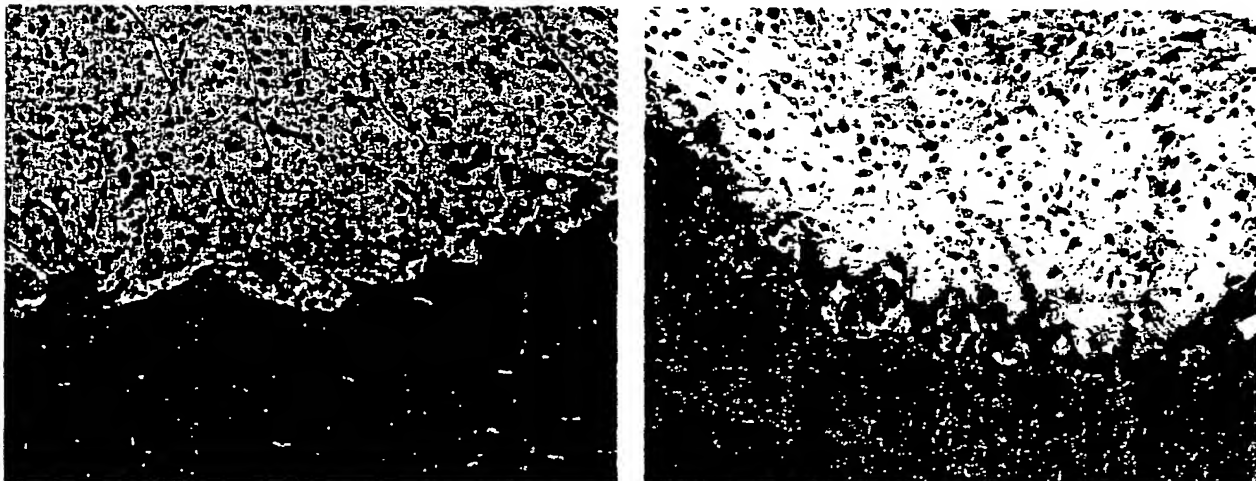
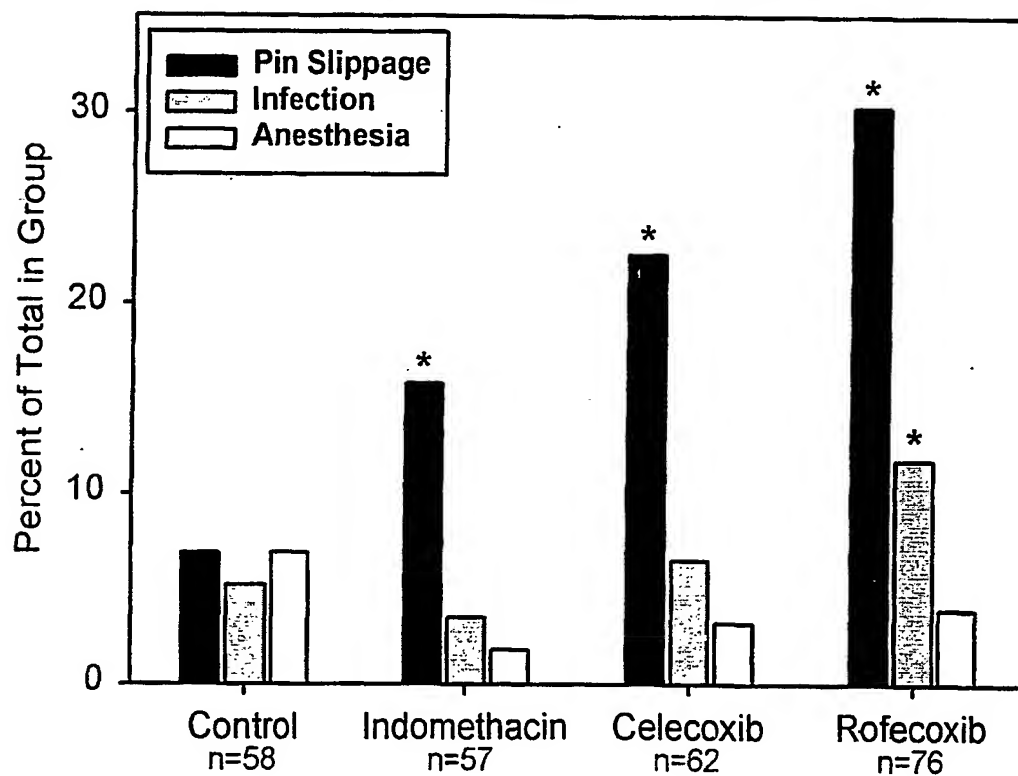


Figure 5. Complication Rates Associated with NSAID Treatment during Fracture Healing (\* indicates  $P < 0.01$  based upon  $\chi^2$ )



# Figure 6. Fracture Healing in Cox Knock-out Mice.

**A.  $Cox1^{-/-}$**



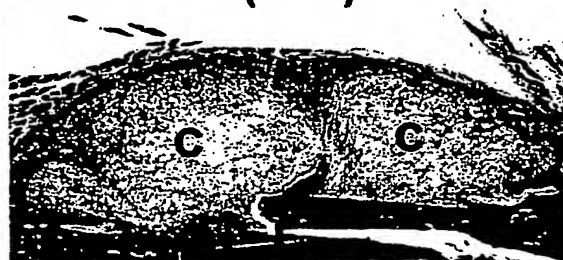
**D.  $Cox2^{-/-}$**



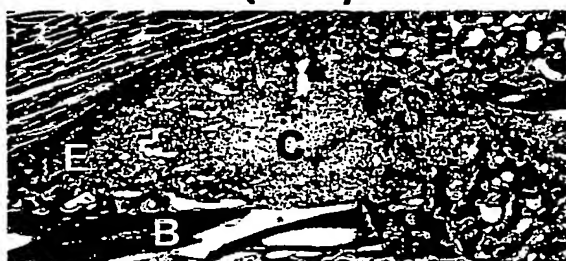
**B.  $Cox1^{-/-}$  (5X)**



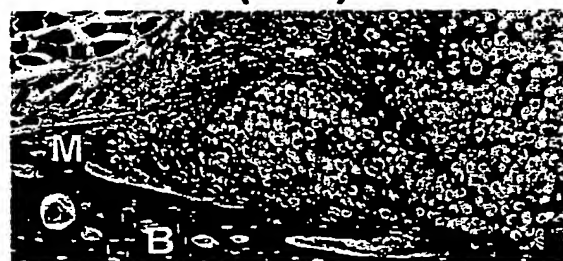
**E.  $Cox2^{-/-}$  (10X)**



**C.  $Cox1^{-/-}$  (10X)**



**F.  $Cox2^{-/-}$  (25X)**



Mouse genotypes are indicated. Panels A and D. Radiographs of mouse femur fractures at 2 weeks post-fracture. Panels B, C, E, and F. Decalcified paraffin sections from the mouse fractures at 2 weeks post-fracture stained with Masson's trichrome. B=cortical bone; F= fracture site; C= chondrocytes and cartilage; E= area of endochondral ossification; M= intramembraneous bone formation. Original photographic magnification is indicated.

267\_043PCT.ST25  
SEQUENCE LISTING

<110> University of Medicine & Dentistry of New Jersey

<120> COX-2 FUNCTION AND WOUND HEALING

<130> 267/043

<140> to be assigned

<141> 2002-09-11

<150> US 09/953,067

<151> 2002-09-10

<160> 8

<170> PatentIn version 3.1

<210> 1

<211> 2554

<212> DNA

<213> Artificial sequence

<220>

<223> Human COX-1 cDNA

<400> 1

gcgccatgag ccggagtctc ttgctccggt tcttgctggt cctgctcctg ctcccgcgcg	60
tcccgcgtcct gctcgcggaac ccagggggcgc ccacgccagt gaatccctgt tgttactatc	120
catgccagca ccagggcatc tgtgtccgct tcggccttga ccgctaccag tgtgactgca	180
cccgcacggg ctattccggc cccaactgca ccatccctgg cctgtggacc tggctccgga	240
attcactgcg gccagcccc tctttcaccc acttcctgct cactcacggg cgctggttct	300
gggagtttgt caatgccacc ttcacccgag agatgctcat gcgcctggta ctacacgtgc	360
gctccaacct tatccccagt cccccacct acaactcagc acatgactac atcagctggg	420
agtctttctc caacgtgagc tattacactc gtattctgcc ctctgtgcct aaagattgcc	480
ccacacccat gggaaccaa gggaagaagc agttgccaga tgcccagctc ctggcccgcg	540
gcttcctgct caggaggaag ttcatacctg accccaagg caccaacctc atgtttgcct	600
tctttgcaca acacttcacc caccagttct tcaaaacttc tggcaagatg ggtcctggct	660

## 267\_043PCT.ST25

tcaccaaggc	cttggggccat	ggggtagacc	tcggccacat	ttatggagac	aatctggagc	720
gtcagtatca	actgcggctc	tttaaggatg	ggaaactcaa	gtaccagggtg	ctggatggag	780
aaatgtaccc	gccctcggta	gaagaggcgc	ctgtgttgat	gcactacccc	cgaggcatcc	840
cgccccagag	ccagatggct	gtgggcccagg	aggtgtttgg	gctgcttcct	gggctcatgc	900
tgtatgccac	gctctggcta	cgtgagcaca	accgtgtgtg	tgacctgctg	aaggctgagc	960
accccacctg	gggcgatgag	cagcttttcc	agacgacccg	cctcatcctc	ataggggaga	1020
ccatcaagat	tgtcatcgag	gagtacgtgc	agcagctgag	tggctatttc	ctgcagctga	1080
aatttgaccc	agagctgctg	ttcgggtgtcc	agttccaata	ccgcaaccgc	attgccatgg	1140
agttcaacca	tctctaccac	tggcaccccc	tcatgcctga	ctccttcaag	gtgggctccc	1200
aggagtacag	ctacgagcag	ttcttgttca	acacctccat	gttggtggac	tatggggttg	1260
aggccctggt	ggatgccttc	tctcgccaga	ttgctggccg	gatcggtggg	ggcaggaaca	1320
tggaccacca	catcctgcat	gtggctgtgg	atgtcatcag	ggagtctcgg	gagatgcggc	1380
tgcagccctt	caatgagtac	cgcaagaggt	ttggcatgaa	accctacacc	tccttccagg	1440
agctcgtagg	agagaaggag	atggcagcag	agttggagga	attgtatgga	gacattgatg	1500
cgttggagtt	ctaccctgga	ctgcttcttg	aaaagtgcc	tccaaactct	atctttgggg	1560
agagtatgat	agagattggg	gctccctttt	ccctcaaggg	tctcctaggg	aatcccatct	1620
gttctccgga	gtactggaag	ccgagcacat	ttggcggcga	ggtgggcttt	aacattgtca	1680
agacggccac	actgaagaag	ctggtctgcc	tcaacaccaa	gacctgtccc	tacgtttcct	1740
tccgtgtgcc	ggatgccagt	caggatgatg	ggcctgctgt	ggagcgacca	tccacagagc	1800
tctgaggggc	aggaaagcag	cattctggag	gggagagctt	tgtgcttgtc	attccagagt	1860
gctgaggcca	gggctgatgg	tcttaaagtgc	tcattttctg	gtttggcatg	gtgagtgttg	1920
gggttgacat	ttagaacttt	aagtctcacc	cattatctgg	aatattgtga	ttctgtttat	1980
tcttccagaa	tgctgaactc	cttgttagcc	cttcagattg	ttaggagtgg	ttctcatttg	2040
gtctgccaga	atactggggt	cttagttgac	aacctagaat	gtcagatttc	tggttgattt	2100

## 267\_043PCT.ST25

gtaacacagt cattctagga tgtggagcta ctgatgaaat ctgctagaaa gttaggggggt 2160  
 tcttattttg cattccagaa tcttgacttt ctgattgggtg attcaaagtg ttgtgttccc 2220  
 tggctgatga tccagaacag tggctcgtat cccaaatctg tcagcatctg gctgtctaga 2280  
 atgtggattt gattcatttt cctgttcagt gagatatcat agagacggag atcctaaggt 2340  
 ccaacaagaa tgcattccct gaatctgtgc ctgcactgag agggcaagga agtgggggtgt 2400  
 tcttcttggg acccccacta agaccctggg ctgaggatgt agagagaaca ggtgggctgt 2460  
 attcacgcca ttggttgga gctaccagag ctctatcccc atccaggtct tgactcatgg 2520  
 cagctgtttc tcatgaagct aataaaattc gcc 2554

<210> 2  
 <211> 4465  
 <212> DNA  
 <213> Artificial sequence

<220>  
 <223> Human COX-2 cDNA

<400> 2  
 caattgtcat acgacttgca gtgagcgtca ggagcacgtc caggaactcc tcagcagcgc 60  
 ctcttctcagc tccacagcca gacgccctca gacagcaaag cctacccccg cgccgcgccc 120  
 tgcccgccgc tcggatgctc gccgcgccc tgctgctgtg cgcggtcctg gcgctcagcc 180  
 atacagcaaa tcttgctgt tcccacccat gtcaaaaccg aggtgtatgt atgagtgtgg 240  
 gatttgacca gtataagtgc gattgtaccc ggacaggatt ctatggagaa aactgctcaa 300  
 caccggaatt ttgacaaga ataaaattat ttctgaaacc cactccaaac acagtgcact 360  
 acatacttac ccacttcaag ggattttgga acgttgtgaa taacattccc ttccttcgaa 420  
 atgcaattat gagttatgtc ttgacatcca gatcacattt gattgacagt ccaccaactt 480  
 acaatgctga ctatggctac aaaagctggg aagccttctc taacctctcc tattatacta 540  
 gagcccttcc tcctgtgcct gatgattgcc cgactccctt ggggtgtcaaa ggtaaaaagc 600  
 agcttcctga ttcaaagag attgtggaaa aattgcttct aagaagaaag ttcattccctg 660

## 267\_043PCT.ST25

atccccaggg	ctcaaacatg	atgtttgcat	tctttgccca	gcacttcacg	catcagtttt	720
tcaagacaga	tcataagcga	gggccagctt	tcaccaacgg	gctgggccat	ggggtggact	780
taaatcatat	ttacggtgaa	actctggcta	gacagcgtaa	actgcgcctt	ttcaaggatg	840
gaaaaatgaa	atatcagata	attgatggag	agatgtatcc	tcccacagtc	aaagatactc	900
aggcagagat	gatctaccct	cctcaagtcc	ctgagcatct	acggtttgct	gtggggcagg	960
aggtctttgg	tctggtgcct	ggtctgatga	tgtatgccac	aatctggctg	cgggaaacaca	1020
acagagtatg	cgatgtgctt	aaacaggagc	atcctgaatg	gggtgatgag	cagttgttcc	1080
agacaagcag	gctaatactg	ataggagaga	ctattaagat	tgtgattgaa	gattatgtgc	1140
aacacttgag	tggctatcac	ttcaaactga	aatttgaccc	agaactactt	ttcaacaaac	1200
aattccagta	ccaaaatcgt	attgctgctg	aatttaacac	cctctatcac	tggcatcccc	1260
ttctgcctga	cacctttcaa	attcatgacc	agaaatacaa	ctatcaacag	tttatctaca	1320
acaactctat	attgctggaa	catggaatta	cccagtttgt	tgaatcattc	accaggcaaa	1380
ttgctggcag	ggttgctggt	ggtaggaatg	ttccaccgcg	agtacagaaa	gtatcacagg	1440
cttccattga	ccagagcagg	cagatgaaat	accagtcttt	taatgagtac	cgcaaacgct	1500
ttatgctgaa	gccctatgaa	tcatttgaag	aacttacagg	agaaaaggaa	atgtctgcag	1560
agttggaagc	actctatggt	gacatcgatg	ctgtggagct	gtatcctgcc	cttctggtag	1620
aaaagcctcg	gccagatgcc	atctttggtg	aaaccatggt	agaagttgga	gcaccattct	1680
ccttgaaagg	acttatgggt	aatgttatat	gttctcctgc	ctactggaag	ccaagcactt	1740
ttggtggaga	agtgggtttt	caaatcatca	acactgcctc	aattcagtct	ctcatctgca	1800
ataacgtgaa	gggctgtccc	tttacttcat	tcagtgttcc	agatccagag	ctcattaata	1860
cagtcaccat	caatgcaagt	tcttcccgtc	ccggactaga	tgatatcaat	cccacagtac	1920
tactaaaaga	acgttcgact	gaactgtaga	agtctaata	tcataatttat	ttatttatat	1980
gaaccatgtc	tattaattta	attatttaat	aatatttata	ttaaactcct	tatgttactt	2040
aacatcttct	gtaacagaag	tcagtactcc	tgttgcggag	aaaggagtca	tacttgtgaa	2100

## 267\_043PCT.ST25

gacttttatg	tcactactct	aaagattttg	ctgttgctgt	taagtttgga	aaacagtttt	2160
tattctgttt	tataaaccag	agagaaatga	gttttgacgt	ctttttactt	gaatttcaac	2220
ttatattata	agaacgaaag	taaagatggt	tgaatactta	aacactatca	caagatggca	2280
aaatgctgaa	agttttttaca	ctgtcgatgt	ttccaatgca	tcttccatga	tgcattagaa	2340
gtaactaatg	tttgaaattt	taaagtactt	ttgggttattt	ttctgtcatc	aaacaaaaac	2400
aggtatcagt	gcattattaa	atgaatattt	aaattagaca	ttaccagtaa	tttcatgtct	2460
actttttaaa	atcagcaatg	aaacaataat	ttgaaatttc	taaattcata	gggtagaatc	2520
acctgtaaaa	gcttgtttga	tttcttaaag	ttattaaact	tgtacatata	ccaaaaagaa	2580
gctgtcttgg	atttaaactct	gtaaaatcag	atgaaatttt	actacaattg	cttgttaaaa	2640
tattttataa	gtgatgttcc	tttttcacca	agagtataaa	ccttttttagt	gtgactgtta	2700
aaacttcctt	ttaaatcaaa	atgccaaatt	tattaagggtg	gtggagccac	tgcagtgtta	2760
tctcaaaaata	agaatatattt	gttgagatat	tccagaattt	gtttatatgg	ctggtaacat	2820
gtaaaatcta	tatcagcaaa	agggtctacc	tttaaaataa	gcaataacaa	agaagaaaac	2880
caaattattg	ttcaaattta	ggttttaaact	tttgaagcaa	actttttttt	atccttgtgc	2940
actgcaggcc	tggtactcag	attttgctat	gaggttaatg	aagtaccaag	ctgtgcttga	3000
ataacgatat	gttttctcag	attttctggt	gtacagttta	atttagcagt	ccatatcaca	3060
ttgcaaaagt	agcaatgacc	tcataaaata	cctcttcaaa	atgcttaaat	tcatttcaca	3120
cattaatttt	atctcagtct	tgaagccaat	tcagtaggtg	cattggaatc	aagcctggct	3180
acctgcatgc	tgttcctttt	cttttcttct	tttagccatt	ttgctaagag	acacagtctt	3240
ctcatcactt	cgtttctcct	attttgtttt	actagtttta	agatcagagt	tcactttctt	3300
tggactctgc	ctatattttc	ttacctgaac	ttttgcaagt	tttcaggtaa	acctcagctc	3360
aggactgcta	tttagctcct	cttaagaaga	ttaaaagaga	aaaaaaaaag	cccttttaaa	3420
aatagtatac	acttatttta	agtgaaaagc	agagaatttt	atttatagct	aatttttagct	3480
atctgtaacc	aagatggatg	caaagaggct	agtgccctcag	agagaactgt	acgggggtttg	3540



## 267\_043PCT.ST25

tgactggaaa aagttacgtt cccattctaa ttaatgccct ttcttattta aaaacaaaac 3600  
 caaatgatat ctaagtagtt ctcagcaata ataataatga cgataatact tcttttccac 3660  
 atctcattgt cactgacatt taatgggtact gtatattact taatttattg aagattatta 3720  
 tttatgtctt attaggacac tatgggtata aactgtgttt aagcctacaa tcattgattt 3780  
 ttttttgtta tgtcacaatc agtatatfff ctttgggggtt acctctctga atattatgta 3840  
 aacaatccaa agaaatgatt gtattaagat ttgtgaataa atttttagaa atctgattgg 3900  
 catattgaga tatttaaggt tgaatgtttg tccttaggat aggcctatgt gctagcccac 3960  
 aaagaatatt gtctcattag cctgaatgtg ccataagact gaccttttaa aatgttttga 4020  
 gggatctgtg gatgcttcgt taatttggtc agccacaatt tattgagaaa atattctgtg 4080  
 tcaagcactg tgggttttaa tttttttaa tcaaacgctg attacagata atagtattta 4140  
 tataaataat tgaaaaaaat tttcttttgg gaagaggag aaaatgaaat aaatatcatt 4200  
 aaagataact caggagaatc ttctttacaa ttttacgttt agaatgttta aggttaagaa 4260  
 agaaatagtc aatatgcttg tataaaacac tgttcactgt tttttttaa aaaaaactt 4320  
 gatttgttat taacattgat ctgctgacaa aacctgggaa tttgggttgt gtatgcgaat 4380  
 gtttcagtgc ctcagacaaa tgtgtattta acttatgtaa aagataagtc tggaaataaa 4440  
 tgtctgttta tttttgtact attta 4465

<210> 3  
 <211> 599  
 <212> PRT  
 <213> Homo sapiens

<400> 3

Met Ser Arg Ser Leu Leu Leu Arg Phe Leu Leu Phe Leu Leu Leu Leu  
 1 5 10 15

Pro Pro Leu Pro Val Leu Leu Ala Asp Pro Gly Ala Pro Thr Pro Val  
 20 25 30

## 267\_043PCT.ST25

Asn Pro Cys Cys Tyr Tyr Pro Cys Gln His Gln Gly Ile Cys Val Arg  
           35                          40                          45

Phe Gly Leu Asp Arg Tyr Gln Cys Asp Cys Thr Arg Thr Gly Tyr Ser  
       50                          55                          60

Gly Pro Asn Cys Thr Ile Pro Gly Leu Trp Thr Trp Leu Arg Asn Ser  
   65                          70                          75                          80

Leu Arg Pro Ser Pro Ser Phe Thr His Phe Leu Leu Thr His Gly Arg  
                           85                          90                          95

Trp Phe Trp Glu Phe Val Asn Ala Thr Phe Ile Arg Glu Met Leu Met  
                   100                          105                          110

Arg Leu Val Leu Thr Val Arg Ser Asn Leu Ile Pro Ser Pro Pro Thr  
           115                          120                          125

Tyr Asn Ser Ala His Asp Tyr Ile Ser Trp Glu Ser Phe Ser Asn Val  
       130                          135                          140

Ser Tyr Tyr Thr Arg Ile Leu Pro Ser Val Pro Lys Asp Cys Pro Thr  
   145                          150                          155                          160

Pro Met Gly Thr Lys Gly Lys Lys Gln Leu Pro Asp Ala Gln Leu Leu  
                           165                          170                          175

Ala Arg Arg Phe Leu Leu Arg Arg Lys Phe Ile Pro Asp Pro Gln Gly  
           180                          185                          190

Thr Asn Leu Met Phe Ala Phe Phe Ala Gln His Phe Thr His Gln Phe  
       195                          200                          205

Phe Lys Thr Ser Gly Lys Met Gly Pro Gly Phe Thr Lys Ala Leu Gly  
       210                          215                          220

## 267\_043PCT.ST25

His Gly Val Asp Leu Gly His Ile Tyr Gly Asp Asn Leu Glu Arg Gln  
 225 230 235 240

Tyr Gln Leu Arg Leu Phe Lys Asp Gly Lys Leu Lys Tyr Gln Val Leu  
 245 250 255

Asp Gly Glu Met Tyr Pro Pro Ser Val Glu Glu Ala Pro Val Leu Met  
 260 265 270

His Tyr Pro Arg Gly Ile Pro Pro Gln Ser Gln Met Ala Val Gly Gln  
 275 280 285

Glu Val Phe Gly Leu Leu Pro Gly Leu Met Leu Tyr Ala Thr Leu Trp  
 290 295 300

Leu Arg Glu His Asn Arg Val Cys Asp Leu Leu Lys Ala Glu His Pro  
 305 310 315 320

Thr Trp Gly Asp Glu Gln Leu Phe Gln Thr Thr Arg Leu Ile Leu Ile  
 325 330 335

Gly Glu Thr Ile Lys Ile Val Ile Glu Glu Tyr Val Gln Gln Leu Ser  
 340 345 350

Gly Tyr Phe Leu Gln Leu Lys Phe Asp Pro Glu Leu Leu Phe Gly Val  
 355 360 365

Gln Phe Gln Tyr Arg Asn Arg Ile Ala Met Glu Phe Asn His Leu Tyr  
 370 375 380

His Trp His Pro Leu Met Pro Asp Ser Phe Lys Val Gly Ser Gln Glu  
 385 390 395 400

Tyr Ser Tyr Glu Gln Phe Leu Phe Asn Thr Ser Met Leu Val Asp Tyr  
 405 410 415

## 267\_043PCT.ST25

Gly Val Glu Ala Leu Val Asp Ala Phe Ser Arg Gln Ile Ala Gly Arg  
                   420                  425                  430

Ile Gly Gly Gly Arg Asn Met Asp His His Ile Leu His Val Ala Val  
                   435                  440                  445

Asp Val Ile Arg Glu Ser Arg Glu Met Arg Leu Gln Pro Phe Asn Glu  
           450                  455                  460

Tyr Arg Lys Arg Phe Gly Met Lys Pro Tyr Thr Ser Phe Gln Glu Leu  
 465                  470                  475                  480

Val Gly Glu Lys Glu Met Ala Ala Glu Leu Glu Glu Leu Tyr Gly Asp  
                   485                  490                  495

Ile Asp Ala Leu Glu Phe Tyr Pro Gly Leu Leu Leu Glu Lys Cys His  
                   500                  505                  510

Pro Asn Ser Ile Phe Gly Glu Ser Met Ile Glu Ile Gly Ala Pro Phe  
           515                  520                  525

Ser Leu Lys Gly Leu Leu Gly Asn Pro Ile Cys Ser Pro Glu Tyr Trp  
           530                  535                  540

Lys Pro Ser Thr Phe Gly Gly Glu Val Gly Phe Asn Ile Val Lys Thr  
 545                  550                  555                  560

Ala Thr Leu Lys Lys Leu Val Cys Leu Asn Thr Lys Thr Cys Pro Tyr  
                   565                  570                  575

Val Ser Phe Arg Val Pro Asp Ala Ser Gln Asp Asp Gly Pro Ala Val  
           580                  585                  590

Glu Arg Pro Ser Thr Glu Leu  
           595

267\_043PCT.ST25

<210> 4  
 <211> 604  
 <212> PRT  
 <213> Homo sapiens

<400> 4

Met Leu Ala Arg Ala Leu Leu Leu Cys Ala Val Leu Ala Leu Ser His  
 1 5 10 15

Thr Ala Asn Pro Cys Cys Ser His Pro Cys Gln Asn Arg Gly Val Cys  
 20 25 30

Met Ser Val Gly Phe Asp Gln Tyr Lys Cys Asp Cys Thr Arg Thr Gly  
 35 40 45

Phe Tyr Gly Glu Asn Cys Ser Thr Pro Glu Phe Leu Thr Arg Ile Lys  
 50 55 60

Leu Phe Leu Lys Pro Thr Pro Asn Thr Val His Tyr Ile Leu Thr His  
 65 70 75 80

Phe Lys Gly Phe Trp Asn Val Val Asn Asn Ile Pro Phe Leu Arg Asn  
 85 90 95

Ala Ile Met Ser Tyr Val Leu Thr Ser Arg Ser His Leu Ile Asp Ser  
 100 105 110

Pro Pro Thr Tyr Asn Ala Asp Tyr Gly Tyr Lys Ser Trp Glu Ala Phe  
 115 120 125

Ser Asn Leu Ser Tyr Tyr Thr Arg Ala Leu Pro Pro Val Pro Asp Asp  
 130 135 140

Cys Pro Thr Pro Leu Gly Val Lys Gly Lys Lys Gln Leu Pro Asp Ser  
 145 150 155 160

## 267\_043PCT.ST25

Asn Glu Ile Val Glu Lys Leu Leu Leu Arg Arg Lys Phe Ile Pro Asp  
 165 170 175

Pro Gln Gly Ser Asn Met Met Phe Ala Phe Phe Ala Gln His Phe Thr  
 180 185 190

His Gln Phe Phe Lys Thr Asp His Lys Arg Gly Pro Ala Phe Thr Asn  
 195 200 205

Gly Leu Gly His Gly Val Asp Leu Asn His Ile Tyr Gly Glu Thr Leu  
 210 215 220

Ala Arg Gln Arg Lys Leu Arg Leu Phe Lys Asp Gly Lys Met Lys Tyr  
 225 230 235 240

Gln Ile Ile Asp Gly Glu Met Tyr Pro Pro Thr Val Lys Asp Thr Gln  
 245 250 255

Ala Glu Met Ile Tyr Pro Pro Gln Val Pro Glu His Leu Arg Phe Ala  
 260 265 270

Val Gly Gln Glu Val Phe Gly Leu Val Pro Gly Leu Met Met Tyr Ala  
 275 280 285

Thr Ile Trp Leu Arg Glu His Asn Arg Val Cys Asp Val Leu Lys Gln  
 290 295 300

Glu His Pro Glu Trp Gly Asp Glu Gln Leu Phe Gln Thr Ser Arg Leu  
 305 310 315 320

Ile Leu Ile Gly Glu Thr Ile Lys Ile Val Ile Glu Asp Tyr Val Gln  
 325 330 335

His Leu Ser Gly Tyr His Phe Lys Leu Lys Phe Asp Pro Glu Leu Leu  
 340 345 350

267\_043PCT.ST25

Phe Asn Lys Gln Phe Gln Tyr Gln Asn Arg Ile Ala Ala Glu Phe Asn  
 355 360 365

Thr Leu Tyr His Trp His Pro Leu Leu Pro Asp Thr Phe Gln Ile His  
 370 375 380

Asp Gln Lys Tyr Asn Tyr Gln Gln Phe Ile Tyr Asn Asn Ser Ile Leu  
 385 390 395 400

Leu Glu His Gly Ile Thr Gln Phe Val Glu Ser Phe Thr Arg Gln Ile  
 405 410 415

Ala Gly Arg Val Ala Gly Gly Arg Asn Val Pro Pro Ala Val Gln Lys  
 420 425 430

Val Ser Gln Ala Ser Ile Asp Gln Ser Arg Gln Met Lys Tyr Gln Ser  
 435 440 445

Phe Asn Glu Tyr Arg Lys Arg Phe Met Leu Lys Pro Tyr Glu Ser Phe  
 450 455 460

Glu Glu Leu Thr Gly Glu Lys Glu Met Ser Ala Glu Leu Glu Ala Leu  
 465 470 475 480

Tyr Gly Asp Ile Asp Ala Val Glu Leu Tyr Pro Ala Leu Leu Val Glu  
 485 490 495

Lys Pro Arg Pro Asp Ala Ile Phe Gly Glu Thr Met Val Glu Val Gly  
 500 505 510

Ala Pro Phe Ser Leu Lys Gly Leu Met Gly Asn Val Ile Cys Ser Pro  
 515 520 525

Ala Tyr Trp Lys Pro Ser Thr Phe Gly Gly Glu Val Gly Phe Gln Ile  
 530 535 540

## 267\_043PCT.ST25

Ile Asn Thr Ala Ser Ile Gln Ser Leu Ile Cys Asn Asn Val Lys Gly  
 545 550 555 560

Cys Pro Phe Thr Ser Phe Ser Val Pro Asp Pro Glu Leu Ile Lys Thr  
 565 570 575

Val Thr Ile Asn Ala Ser Ser Ser Arg Ser Gly Leu Asp Asp Ile Asn  
 580 585 590

Pro Thr Val Leu Leu Lys Glu Arg Ser Thr Glu Leu  
 595 600

<210> 5  
 <211> 2757  
 <212> DNA  
 <213> Artificial sequence

<220>  
 <223> Mouse COX-1 cDNA

<400> 5  
 gccgttggca ttgcacatcc atccactccc agagtcacga gtcgaaggag tctctcgctc 60  
 tgggtttcccc tgctgctgct cctgctgctg ccgccgacac cctcgggtcct gctcgcagat 120  
 cctgggggtgc cctcaccagt caatccctgt tgttactatc cgtgccagaa ccaggggtgtc 180  
 tgtgtccgct ttggcctcga caactaccag tgtgattgta ctgcacaggg ctactcaggc 240  
 cccaactgta ccacccctga gatctggacc tggcttcgga attctctgcg gccagcccc 300  
 tcgttcaccc atttctgct gacacatgga tactggctct gggaatttgt gaatgccacc 360  
 ttcacccgag aagtactcat gcgcctggta ctacacgtgc ggtccaacct tatccccagc 420  
 cctccgacct acaactcagc gcatgactac atcagctggg agtccttctc caatgtgagc 480  
 tactatactc gcattctgcc ctctgtaccc aaagactgcc ccacacccat ggggacccaa 540  
 gggaagaaac agttaccaga tgttcagctt ctggcccaac agctgctgct gagaagggag 600  
 ttcattcctg cccccaggg caccaacatc ctgtttgcct tctttgcaca acacttcacc 660  
 caccagttct tcaagacctc tggaaagatg ggtcctgggt ttaccaaggc cttaggccac 720



## 267\_043PCT.ST25

ggggtagacc	ttggccacat	ttatggagat	aatctggaac	gacagtatca	cctgcggctc	780
ttcaaggatg	ggaaacttaa	gtaccagggtg	ctggacggag	aggtgtaccc	accttccgtg	840
gaacaggcgt	ccgtgttgat	gcgctaccca	ccagggtgtcc	cgcctgaaag	gcagatggct	900
gtgggccagg	aggtgttttg	gttgcttccg	gggctgatgc	tcttctccac	gatctggctt	960
cgtgaacata	accgcgtgtg	cgacctgctg	aaggaggagc	atcccacgtg	ggatgatgag	1020
cagctcttcc	agaccactcg	cctcatcctt	ataggagaaa	ccatcaaaat	tgtcattgag	1080
gagtatgtgc	agcacttgag	tggctatttc	ctgcagctca	agtttgaccc	ggagctgctg	1140
ttccgagccc	agttccaata	tcgaaaccgc	atcgccatgg	aatttaacca	tctctatcac	1200
tggcatccac	tcatgcccaa	ctccttccaa	gtgggctcac	aagagtacag	ctacgagcag	1260
tttttattta	acacttctat	gctgggtggac	tatgggggtg	aggcactggg	ggatgccttc	1320
tctcgccaga	gggctggccg	gattggtgga	ggtaggaact	ttgactatca	tgttctgcat	1380
gtggctgtgg	atgtcatcaa	ggagtcccga	gagatgcgcc	tacagccctt	caatgaatac	1440
cgaaagaggt	ttggcttgaa	gccttacacc	tctttccagg	agctcacagg	agagaaggag	1500
atggctgctg	agttggagga	gctgtacggg	gacatcgatg	ctttagagtt	ctacccgggg	1560
ttgctgctgg	agaagtgcc	gcccactcc	atctttggag	aaagtatgat	agagatgggg	1620
gctccctttt	ccctcaaggg	cctcctaggg	aatcccatct	gttcccaga	gtactggaaa	1680
cccagcacgt	tcggtgggtg	cgtgggcttc	aaccttgcca	acacagcctc	actgaagaaa	1740
ctggctctgcc	tcaacaccaa	gacctgcccc	tatgtttcct	tccgtgtgcc	agattaccct	1800
ggagatgacg	ggtctgtcct	agtgagacgc	tccactgagc	tctgaggggag	ctggaaagca	1860
gcctctggag	ggaggagttt	tgttcctgat	gaagacaagt	ccttgatgtg	ggttttcgtg	1920
gcttggcatt	gtgagagctg	atgctcacat	ttgaaacttt	gggtcttacc	cttgccctaga	1980
aaattgtgat	tttgccactt	tcggatgttg	aattctttgt	taactaagaa	agttagaagt	2040
ggttttgtct	gcctcctcag	aacttggctc	tttgttggca	actcagaaaag	tcagatttct	2100
ggttgatttg	gaatataggc	ttaaaacttt	atattatagg	gtaggggtgtg	gttgcacaca	2160

## 267\_043PCT.ST25

ccttaatccc agcacttgga aggcagaggc agttggatct ctgggagttt gaggccagtt 2220  
 tggcctatat agtgagttct aggccagcca tggatgcata gtgagactct ttctcaaaac 2280  
 aaacaaacaa acaaacaaaac aaacaacttt tagaatgtag aattccgtaa aaaaaaaaaa 2340  
 tcccttgaaa gttaatgggg tcctcaattt tcttctctaga atttggaggc ctcttcagaa 2400  
 tgttgactat ctgacaggtg actcagaagg tcctgttcct ggtcaatgat ctataacatg 2460  
 ggccaaaaca ttcccaactt gaatgtctag aatgtggaat tggttcattt tcctgttcag 2520  
 tgaaatggac acagaacaaa agaaccagcgt gtccagcaag aattgccttg cccaaaccta 2580  
 cgtctacgcc aaaggtcaag gcagtaaggc gttcttggga gccacactta gactctttcc 2640  
 aaagatgtgg agggaaacaga tggactcatc tatgatcttg gttggaaacc accacagttc 2700  
 tatccccatc cagatctttg cttgtggcag ctgtttctca tgaagctaata aaaattc 2757

<210> 6  
 <211> 3986  
 <212> DNA  
 <213> Artificial sequence

<220>  
 <223> Mouse COX-2 cDNA

<400> 6  
 agttgtcaaa ctgagagcta agagcttcag gagtcagtca ggactctgct cacgaagaat 60  
 ctcagcactg catcctgcca gctccaccgc caccaccact gccacctccg ctgccacctc 120  
 tgcgatgctc ttccgagctg tgctgctctg cgctgccttg gggctcagcc aggcagcaaa 180  
 tccttgctgt tccaatccat gtcaaaaccg tggggaatgt atgagcacag gatttgacca 240  
 gtataagtgt gactgtaccc ggactggatt ctatggtgaa aactgtacta cacctgaatt 300  
 tctgacaaga atcaaattac tgctgaagcc caccaccaaac acagtgcact acatcctgac 360  
 ccacttcaag ggagtctgga acattgtgaa caacatcccc ttcttgcgaa gtttaactat 420  
 gaaatatgtg ctgacatcca gatcatattt gattgacagt ccacctactt acaatgtgca 480  
 ctatggttac aaaagctggg aagccttctc caacctctcc tactacacca gggcccttcc 540

## 267\_043PCT.ST25

tccagtagca gatgactgcc caactcccat ggggtgtgaag ggaaataagg agcttcctga	600
ttcaaaagaa gtgctggaaa aggttcttct acggagagag ttcattccctg accccaagg	660
ctcaaatatg atgtttgcat tctttgcca gcacttcacc catcagtttt tcaagacaga	720
tcataagcga ggacctgggt tcacccgagg actgggccat ggagtggact taaatcacat	780
ttatggtgaa actctggaca gacaacataa actgcgctt ttcaaggatg gaaaattgaa	840
atatcaggtc attggtggag aggtgtatcc cccacagtc aaagacactc aggtagagat	900
gatctaccct cctcacatcc ctgagaacct gcagtttgct gtggggcagg aagtctttgg	960
tctggtgcct ggtctgatga tgtatgccac catctggctt cgggagcaca acagagtgtg	1020
cgacatactc aagcaggagc atcctgagtg ggggtgatgag caactattcc aaaccagcag	1080
actcatactc ataggagaga ctatcaagat agtgatcgaa gactacgtgc aacacctgag	1140
cggttaccac ttcaaactca agtttgacct agagctcctt ttcaaccagc agttccagta	1200
tcagaaccgc attgcctctg aattcaacac actctatcac tggcaccctc tgctgccga	1260
caccttcaac attgaagacc aggagtacag cttcaaacag tttctctaca acaactccat	1320
cctcctggaa catggactca ctcagtttgt tgagtcattc accagacaga ttgctggccg	1380
ggttgctggg ggaagaaatg tgccaattgc tgtacaagca gtggcaaagg cctccattga	1440
ccagagcaga gagatgaaat accagtctct caatgagtac cgcaaacgct tctccctgaa	1500
gccgtacaca tcatttgaag aacttacagg agagaaggaa atggctgcag aattgaaagc	1560
cctctaagat gacatcgatg tcatggaact gtaccctgcc ctgctgggtg aaaaacctcg	1620
tccagatgct atctttgggg agaccatggt agagcttgga gcaccattct ccttgaaagg	1680
acttatggga aatcccatct gttctcctca atactggaag ccgagcacct ttggaggcga	1740
agtgggtttt aagatcatca atactgcctc aattcagtct ctcatctgca ataattgtgaa	1800
ggggtgtccc ttcacttctt tcaatgtgca agatccacag cctacaaaaa cagccacct	1860
caatgcaagt gcctcccact ccagactaga tgacattaac cctacagtac taatcaaaag	1920
gcgttcaact gagctgtaaa agtctactga ccatatttat ttatttatgt gaaggaattt	1980

## 267\_043PCT.ST25

aatttaatta	tttaatat	ttt atactgaatt	ttttttcatg	taacatcttc	cataacagaa	2040
ggcaatgttc	ttgaacaatg	ttacatttgt	gaagattcct	ccggtgtttg	tcctttaaat	2100
atgtgttacc	tgaaactgaa	aggaaatcag	cattcattcc	tctacataag	ccagtgagaa	2160
gggaaatgaa	ttttgatatc	tttatacttg	aatttcagat	catgaattag	cttaacaaga	2220
accaaggaaa	aatgtatgaa	tatgtgagtg	ttgtttacaag	atgaaaaatg	ctgcaggtat	2280
caacactgtt	ggttacactg	tgtcttcttt	acctatgata	ggagcatgta	atgtggaatt	2340
cgtcttaaat	ccttgcatat	ctttatctca	tcaaacaag	gggtccaagt	tcagttttaa	2400
ataagcattt	aaggcagata	ctgacaacaa	tctcattttt	ttaaaatgtt	gtcttgagac	2460
aaataatttg	aaatttctaa	attgggacgt	ttgaatcact	tttgaaagct	cttactttct	2520
taagctgtca	ggtttgtacc	gacatggagt	aaacagctat	cataaacgta	aatctccaaa	2580
actagtagaa	attatgtcat	gattgatggg	taagatacca	tgtcagggat	tgtcttttct	2640
tagaagtagt	gaaagctact	tactatgaca	atcagacctt	ccttgtagtg	caaaatgctg	2700
gtgtggaagg	tggtggagcc	cgtgctgctc	tgtcttaact	atgagtgtga	gctttaaagc	2760
tcgttgatga	gtggtagcca	gcaaagccta	gagcaacaaa	agcgttctac	aaaggaacta	2820
accaagaaca	aagaagggtt	cccaattaaa	gatcacattc	agggttaaac	ttccaaagga	2880
gacatcctga	tcctgggttt	gtgctggcct	ggtactcagt	aggtttttgc	tgtgaggtta	2940
aagacttgcc	agggtgaac	ttcgaaacag	tttttctggt	gcacagtatg	atgtaacagt	3000
ccatctctca	atgcaatagg	tatcagtggc	ctcgtgagct	tcttcacaat	atttgatatg	3060
tcttccagcc	cattgaacct	ggactgcaga	aggcccatg	tcagtgtgta	gctcagcctg	3120
gatgccagca	tttgctgctc	ctcttagttc	cgtttctcgt	ggtcacttta	ctacgagaaa	3180
cgctgattgg	gttttcgtag	ctgtgttacc	aggtttttag	tatcagaact	attcttttct	3240
taacctctat	tcataatttt	tctacttgaa	gttttacatt	caggaaaacc	tcagcgctca	3300
ggactactat	gtacctcccc	tttgaggga	aaaattcatt	tttaggtaaa	aggcaaaaat	3360
tttttaaaaa	tattttttat	ttataattat	atggaagggc	cctaccaaga	tgctagaaat	3420

267\_043PCT.ST25

atagggagtt cctgacaaga aatttccatt cttattctga agaattgctt tcttacttaa 3480  
 aaacaaagac agtttgtgag tagttctggg caatagggat aaatataaaa caataatgat 3540  
 gatcattttc tacatctcat tatcagctga ggtactgtat attactgaat ttattgaaga 3600  
 tagttatgtc ttttagacat tgttgttata aactatgttt aagcctacta caagtgtttc 3660  
 ttttttgcac tatgttggaa ttgatgtacc ttttttatga ttacctctct gaactatggt 3720  
 gtgaacaatc aaacaaaatg atgagattaa cgttcatgga taaattctaa gaaaactagt 3780  
 gtattttttt gaaaagtttg aagttagaac ttaggctggt ggaatttacg cataaagcag 3840  
 actgcatagg atccaatatt gactgacca agcatgttat aaagactgac attttagaca 3900  
 ttttgaaggc cctgtaagtg tttattaatt agttagaact taattgatta aaaaatatat 3960  
 ccaaagcact ataggcatta gaattc 3986

<210> 7  
 <211> 602  
 <212> PRT  
 <213> Mus musculus

<400> 7

Met Ser Arg Arg Ser Leu Ser Leu Trp Phe Pro Leu Leu Leu Leu Leu  
1 5 10 15

Leu Leu Pro Pro Thr Pro Ser Val Leu Leu Ala Asp Pro Gly Val Pro  
20 25 30

Ser Pro Val Asn Pro Cys Cys Tyr Tyr Pro Cys Gln Asn Gln Gly Val  
35 40 45

Cys Val Arg Phe Gly Leu Asp Asn Tyr Gln Cys Asp Cys Thr Arg Thr  
50 55 60

Gly Tyr Ser Gly Pro Asn Cys Thr Ile Pro Glu Ile Trp Thr Trp Leu  
65 70 75 80

## 267\_043PCT.ST25

Arg Asn Ser Leu Arg Pro Ser Pro Ser Phe Thr His Phe Leu Leu Thr  
                             85                            90                            95

His Gly Tyr Trp Leu Trp Glu Phe Val Asn Ala Thr Phe Ile Arg Glu  
                             100                            105                            110

Val Leu Met Arg Leu Val Leu Thr Val Arg Ser Asn Leu Ile Pro Ser  
                             115                            120                            125

Pro Pro Thr Tyr Asn Ser Ala His Asp Tyr Ile Ser Trp Glu Ser Phe  
                             130                            135                            140

Ser Asn Val Ser Tyr Tyr Thr Arg Ile Leu Pro Ser Val Pro Lys Asp  
                             145                            150                            155                            160

Cys Pro Thr Pro Met Gly Thr Lys Gly Lys Lys Gln Leu Pro Asp Val  
                             165                            170                            175

Gln Leu Leu Ala Gln Gln Leu Leu Leu Arg Arg Glu Phe Ile Pro Ala  
                             180                            185                            190

Pro Gln Gly Thr Asn Ile Leu Phe Ala Phe Phe Ala Gln His Phe Thr  
                             195                            200                            205

His Gln Phe Phe Lys Thr Ser Gly Lys Met Gly Pro Gly Phe Thr Lys  
                             210                            215                            220

Ala Leu Gly His Gly Val Asp Leu Gly His Ile Tyr Gly Asp Asn Leu  
                             225                            230                            235                            240

Glu Arg Gln Tyr His Leu Arg Leu Phe Lys Asp Gly Lys Leu Lys Tyr  
                             245                            250                            255

Gln Val Leu Asp Gly Glu Val Tyr Pro Pro Ser Val Glu Gln Ala Ser  
                             260                            265                            270

## 267\_043PCT.ST25

Val Leu Met Arg Tyr Pro Pro Gly Val Pro Pro Glu Arg Gln Met Ala  
                   275                                  280                                  285

Val Gly Gln Glu Val Phe Gly Leu Leu Pro Gly Leu Met Leu Phe Ser  
           290                                  295                                  300

Thr Ile Trp Leu Arg Glu His Asn Arg Val Cys Asp Leu Leu Lys Glu  
   305                                  310                                  315                                  320

Glu His Pro Thr Trp Asp Asp Glu Gln Leu Phe Gln Thr Thr Arg Leu  
                                   325                                  330                                  335

Ile Leu Ile Gly Glu Thr Ile Lys Ile Val Ile Glu Glu Tyr Val Gln  
                   340                                  345                                  350

His Leu Ser Gly Tyr Phe Leu Gln Leu Lys Phe Asp Pro Glu Leu Leu  
                   355                                  360                                  365

Phe Arg Ala Gln Phe Gln Tyr Arg Asn Arg Ile Ala Met Glu Phe Asn  
           370                                  375                                  380

His Leu Tyr His Trp His Pro Leu Met Pro Asn Ser Phe Gln Val Gly  
   385                                  390                                  395                                  400

Ser Gln Glu Tyr Ser Tyr Glu Gln Phe Leu Phe Asn Thr Ser Met Leu  
                                   405                                  410                                  415

Val Asp Tyr Gly Val Glu Ala Leu Val Asp Ala Phe Ser Arg Gln Arg  
                   420                                  425                                  430

Ala Gly Arg Ile Gly Gly Gly Arg Asn Phe Asp Tyr His Val Leu His  
           435                                  440                                  445

Val Ala Val Asp Val Ile Lys Glu Ser Arg Glu Met Arg Leu Gln Pro  
           450                                  455                                  460

## 267\_043PCT.ST25

Phe Asn Glu Tyr Arg Lys Arg Phe Gly Leu Lys Pro Tyr Thr Ser Phe  
 465 470 475 480

Gln Glu Leu Thr Gly Glu Lys Glu Met Ala Ala Glu Leu Glu Glu Leu  
 485 490 495

Tyr Gly Asp Ile Asp Ala Leu Glu Phe Tyr Pro Gly Leu Leu Leu Glu  
 500 505 510

Lys Cys Gln Pro Asn Ser Ile Phe Gly Glu Ser Met Ile Glu Met Gly  
 515 520 525

Ala Pro Phe Ser Leu Lys Gly Leu Leu Gly Asn Pro Ile Cys Ser Pro  
 530 535 540

Glu Tyr Trp Lys Pro Ser Thr Phe Gly Gly Asp Val Gly Phe Asn Leu  
 545 550 555 560

Val Asn Thr Ala Ser Leu Lys Lys Leu Val Cys Leu Asn Thr Lys Thr  
 565 570 575

Cys Pro Tyr Val Ser Phe Arg Val Pro Asp Tyr Pro Gly Asp Asp Gly  
 580 585 590

Ser Val Leu Val Arg Arg Ser Thr Glu Leu  
 595 600

<210> 8  
 <211> 604  
 <212> PRT  
 <213> Mus musculus

<400> 8

Met Leu Phe Arg Ala Val Leu Leu Cys Ala Ala Leu Gly Leu Ser Gln  
 1 5 10 15



## 267\_043PCT.ST25

Ala Ala Asn Pro Cys Cys Ser Asn Pro Cys Gln Asn Arg Gly Glu Cys  
 20 25 30

Met Ser Thr Gly Phe Asp Gln Tyr Lys Cys Asp Cys Thr Arg Thr Gly  
 35 40 45

Phe Tyr Gly Glu Asn Cys Thr Thr Pro Glu Phe Leu Thr Arg Ile Lys  
 50 55 60

Leu Leu Leu Lys Pro Thr Pro Asn Thr Val His Tyr Ile Leu Thr His  
 65 70 75 80

Phe Lys Gly Val Trp Asn Ile Val Asn Asn Ile Pro Phe Leu Arg Ser  
 85 90 95

Leu Thr Met Lys Tyr Val Leu Thr Ser Arg Ser Tyr Leu Ile Asp Ser  
 100 105 110

Pro Pro Thr Tyr Asn Val His Tyr Gly Tyr Lys Ser Trp Glu Ala Phe  
 115 120 125

Ser Asn Leu Ser Tyr Tyr Thr Arg Ala Leu Pro Pro Val Ala Asp Asp  
 130 135 140

Cys Pro Thr Pro Met Gly Val Lys Gly Asn Lys Glu Leu Pro Asp Ser  
 145 150 155 160

Lys Glu Val Leu Glu Lys Val Leu Leu Arg Arg Glu Phe Ile Pro Asp  
 165 170 175

Pro Gln Gly Ser Asn Met Met Phe Ala Phe Phe Ala Gln His Phe Thr  
 180 185 190

His Gln Phe Phe Lys Thr Asp His Lys Arg Gly Pro Gly Phe Thr Arg  
 195 200 205

## 267\_043PCT.ST25

Gly Leu Gly His Gly Val Asp Leu Asn His Ile Tyr Gly Glu Thr Leu  
 210 215 220

Asp Arg Gln His Lys Leu Arg Leu Phe Lys Asp Gly Lys Leu Lys Tyr  
 225 230 235 240

Gln Val Ile Gly Gly Glu Val Tyr Pro Pro Thr Val Lys Asp Thr Gln  
 245 250 255

Val Glu Met Ile Tyr Pro Pro His Ile Pro Glu Asn Leu Gln Phe Ala  
 260 265 270

Val Gly Gln Glu Val Phe Gly Leu Val Pro Gly Leu Met Met Tyr Ala  
 275 280 285

Thr Ile Trp Leu Arg Glu His Asn Arg Val Cys Asp Ile Leu Lys Gln  
 290 295 300

Glu His Pro Glu Trp Gly Asp Glu Gln Leu Phe Gln Thr Ser Arg Leu  
 305 310 315 320

Ile Leu Ile Gly Glu Thr Ile Lys Ile Val Ile Glu Asp Tyr Val Gln  
 325 330 335

His Leu Ser Gly Tyr His Phe Lys Leu Lys Phe Asp Pro Glu Leu Leu  
 340 345 350

Phe Asn Gln Gln Phe Gln Tyr Gln Asn Arg Ile Ala Ser Glu Phe Asn  
 355 360 365

Thr Leu Tyr His Trp His Pro Leu Leu Pro Asp Thr Phe Asn Ile Glu  
 370 375 380

Asp Gln Glu Tyr Ser Phe Lys Gln Phe Leu Tyr Asn Asn Ser Ile Leu  
 385 390 395 400

## 267\_043PCT.ST25

Leu Glu His Gly Leu Thr Gln Phe Val Glu Ser Phe Thr Arg Gln Ile  
 405 410 415

Ala Gly Arg Val Ala Gly Gly Arg Asn Val Pro Ile Ala Val Gln Ala  
 420 425 430

Val Ala Lys Ala Ser Ile Asp Gln Ser Arg Glu Met Lys Tyr Gln Ser  
 435 440 445

Leu Asn Glu Tyr Arg Lys Arg Phe Ser Leu Lys Pro Tyr Thr Ser Phe  
 450 455 460

Glu Glu Leu Thr Gly Glu Lys Glu Met Ala Ala Glu Leu Lys Ala Leu  
 465 470 475 480

Tyr Ser Asp Ile Asp Val Met Glu Leu Tyr Pro Ala Leu Leu Val Glu  
 485 490 495

Lys Pro Arg Pro Asp Ala Ile Phe Gly Glu Thr Met Val Glu Leu Gly  
 500 505 510

Ala Pro Phe Ser Leu Lys Gly Leu Met Gly Asn Pro Ile Cys Ser Pro  
 515 520 525

Gln Tyr Trp Lys Pro Ser Thr Phe Gly Gly Glu Val Gly Phe Lys Ile  
 530 535 540

Ile Asn Thr Ala Ser Ile Gln Ser Leu Ile Cys Asn Asn Val Lys Gly  
 545 550 555 560

Cys Pro Phe Thr Ser Phe Asn Val Gln Asp Pro Gln Pro Thr Lys Thr  
 565 570 575

Ala Thr Ile Asn Ala Ser Ala Ser His Ser Arg Leu Asp Asp Ile Asn  
 580 585 590

267\_043PCT.ST25  
Pro Thr Val Leu Ile Lys Arg Arg Ser Thr Glu Leu  
595 600

(19) World Intellectual Property  
Organization  
International Bureau



(43) International Publication Date  
20 March 2003 (20.03.2003)

PCT

(10) International Publication Number  
**WO 2003/022224 A3**

(51) International Patent Classification<sup>7</sup>: **C12N 15/00**,  
15/09, 15/63, 15/70, 15/74, A01N 43/04, A61K 31/70

(21) International Application Number:  
PCT/US2002/028930

(22) International Filing Date:  
11 September 2002 (11.09.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
09/953,067 11 September 2001 (11.09.2001) US

(71) Applicant (*for all designated States except US*): **UNI-  
VERSITY OF MEDICINE AND DENTISTRY OF  
NEW JERSEY** [US/US]; 335 George Street, Suite 3200,  
New Brunswick, NJ 08901 (US).

(72) Inventor; and

(75) Inventor/Applicant (*for US only*): **O'CONNOR,  
Patrick, J.** [US/US]; 65 Waldon Road, Fanwood, NJ  
07023 (US).

(74) Agents: **WISE, Michael, J. et al.**; Perkins Coie LLP, P.O.  
Box 1208, Seattle, WA 98111-1208 (US).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,  
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,  
CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,  
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,  
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,  
MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG,  
SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ,  
VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM,  
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),  
Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),  
European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE,  
ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK,  
TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ,  
GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report*

(88) Date of publication of the international search report:  
8 January 2004

*For two-letter codes and other abbreviations, refer to the "Guid-  
ance Notes on Codes and Abbreviations" appearing at the begin-  
ning of each regular issue of the PCT Gazette.*

(54) Title: **COX-2 FUNCTION AND WOUND HEALING**

(57) Abstract: The invention relates to compositions and methods for enhancing bone healing, bone formation and wound healing. More specifically, it relates to the use of cyclooxygenase 2 (COX-2) following bone fracture, orthopaedic procedure or wound inflection to enhance healing.



**WO 2003/022224 A3**

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/28930

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C12N 15/00, 15/09, 15/63, 15/70, 15/74; A01N 43/04; A61K 31/70  
US CL : 435/320.1; 514/44

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
U.S. : 435/320.1; 514/44

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
Please See Continuation Sheet

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6,107,087 A (O'NEILL et al) 22 August 2000 (22.08.2000), see whole document, especially abstract and columns 16 and 37-38.	1-4
---		-----
Y		21-22
A	HUANG et al. Gene transfer to cultured human endometrial stromal cells: A model to study cyclooxygenase-2 gene regulation. Fertility and Sterility. October 1998, Vol. 70, No. 4, pages 734-739.	1-4 and 21-22
A	MCCORMACK et al. Roles of COX-1 and COX-2. J. Rheumatol. November 1998, Vol. 25, No. 11, pages 2279-81.	1-4 and 21-22
Y	NEEDLEMAN et al. The discovery and function of COX-2. J. Rheumatol. 1997, Vol. 24, Supplement 49, pages 6-8.	1-4, 21-22
X	WU. Injury-coupled induction of endothelial eNOS and COX-2 genes: A paradigm for thromboresistant gene therapy Proceedings of the Association of American Physicians. 1998, Vol. 110, No. 3, pages 163-170.	1-2
---		-----
Y		3-4, 21-22



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E" earlier application or patent published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

25 November 2002 (25.11.2002)

Date of mailing of the international search report

23 JUL 2003

Name and mailing address of the ISA/US

Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

Facsimile No. (703)305-3230

Authorized officer

*Felicia D. Roberts*  
Brian Whiteman

Telephone No. 703 308-0196

Form PCT/ISA/210 (second sheet) (July 1998)

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/28930

## Box II Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claim Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claim Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box III Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:  
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.: 1-4, 21 and 22
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐  
☒

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

PCT/US02/28930

### BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions, which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s) 1-4, drawn to a vector comprising a promoter operably linked to a cyclooxygenase expression cassette.

Group II, claim(s) 5-12, drawn to a method for enhancing wound healing comprising: delivering the vector of claim 1 to the location of the wound.

Group III, claim(s) 13-14, drawn to a method of treating a pathological heterotopic ossification condition in a patient comprising administering COX-2 to the patient.

Group IV, claim(s) 15-16, drawn to a method for inhibiting wound healing comprising administering an effective amount of NSAIDs to the location of the wound.

Group V, claim(s) 17, drawn to a method of treating osteoporosis and brittle bone conditions in a mammal comprising administering the vector of claim 1 to the mammal.

Group VI, claim(s) 18-20, drawn to a composition for wound healing comprising a formulated cyclooxygenase protein.

Group VII, claim(s) 21-22, drawn to a method for treating wound comprising administering the composition of claim 18 to a patient with a wound.

Group VIII, claim(s) 23, drawn to a method of enhancing wound healing, wherein prostaglandin production is stimulated in at least one osteoblast located at or near the wound.

Group IX, claim(s) 24-25, drawn to a method of enhancing wound healing comprising administering stable prostaglandins to the location of the wound.

The invention listed as Groups I-IX do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding technical feature for the following reasons:

The technical feature linking groups I-IX appears to be that they all relate to a vector comprising a promoter operably linked to a cyclooxygenase expression cassette.

However, O'Neill et al. teaches a vector comprising a promoter operably linked to a cyclooxygenase expression cassette.

Therefore, the technical feature linking the inventions of groups I-IX does not constitute a special technical feature as defined by PCT Rule 13.2, as it does not define a contribution over the prior art.

The special technical feature of Group I is drawn to a vector comprising a promoter operably linked to a cyclooxygenase expression cassette.

The special technical feature of Group II is drawn to a method for enhancing wound healing comprising: delivering the vector of claim 1 to the location of the wound.

The special technical feature of Group III is a method of treating a pathological heterotopic ossification condition in a patient comprising administering COX-2 to the patient.

Form PCT/ISA/210 (second sheet) (July 1998)



## INTERNATIONAL SEARCH REPORT

PCT/US02/28930

The special technical feature of Group IV is drawn to a method for inhibiting wound healing comprising administering an effective amount of NSAIDs to the location of the wound.

The special technical feature of Group V is drawn to a method of treating osteoporosis and brittle bone conditions in a mammal comprising administering the vector of claim 1 to the mammal.

The special technical feature of Group VI is drawn to a composition for wound healing comprising a formulated cyclooxygenase protein.

The special technical feature of Group VII is drawn to a method for treating wound comprising administering the composition of claim 18 to a patient with a wound.

The special technical feature of Group VIII is drawn to a method of enhancing wound healing, wherein prostaglandin production is stimulated in at least one osteoblast located at or near the wound.

The special technical feature of Group IX is drawn to a method of enhancing wound healing comprising administering stable prostaglandins to the location of the wound.

Accordingly, Groups I-IX are not so linked by the same or a corresponding technical feature as to form a single general inventive concept.

### Continuation of B. FIELDS SEARCHED Item 3:

WEST 2.1; STN

search terms: COX-2, wound healing, gene therapy, gene delivery, vector, cyclooxygenase

**THIS PAGE BLANK (USPTO)**

**This Page is Inserted by IFW Indexing and Scanning  
Operations and is not part of the Official Record**

**BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant:

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☒ FADED TEXT OR DRAWING
- ☒ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☐ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: \_\_\_\_\_

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.**

**THIS PAGE BLANK (USPTO)**